Diploma thesis

Management of Fanconi Anemia and other Hematologically and Immunologically relevant Tumor Predisposing Syndromes in Pediatric Hematology Oncology in Austria

Cohort study and Development of a Registry for Chromosome Instability Syndromes

submitted by

Rebecca Voss

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supervised by

Univ. Prof. Dr. Markus Seidel

Univ. Prof. Dr. Martin Benesch

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Affirmation

I hereby affirm that this thesis is my own unaided work. All sources I have used or quoted have been indicated as such and acknowledged by means of complete references in the text.

Graz, January 9th, 2016 *Rebecca Katherina Voss eh.*

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Abstract

Chromosome instability syndromes with DNA-repair defects represent a rare group of childhood diseases with (mainly) autosomal recessive inheritance patterns. The manifestation and severity of these syndromes vary from patient to patient. They often present congenital physical abnormalities, such as microcephaly, skeletal abnormalities, abnormalities of the skin and abnormalities of the urogenital tract among others. Furthermore, progressive bone marrow failure (BMF) or immunodeficiency and predisposition to malignancy are seen in such diseases. On a molecular level, they show cellular hypersensitivity to DNA-cross linking agents and severe radio-sensitivity.

The aim of this study was to determine which syndromes were represented and how many patients were affected, how their disease progressed and what therapies they received.

Information from patients alive in 2000 and later was gathered directly from pediatric hematology-oncology units all over Austria. A database was created to collect patient information and serve as the basis for a future registry for chromosome instability syndromes.

In 47 patients included in this study, four different syndromes were found: Fanconi anemia (FA), Ataxia telangiectasia (ATM), Nijmegen breakage syndrome (NBS) and Artemis deficiency (AD). Other syndromes, such as Bloom syndrome, constitutive mismatch repair deficiency, DNA ligase 4 deficiency or Cernunnos deficiency could not be identified.

All 30 FA patients showed signs of progressive BMF, 70% of patients underwent hematopoietic stem cell transplantation (HSCT). Malignancies were observed in 57% of patients, such as myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and squamous cell carcinoma of head and neck (HNSCC) among others. 33% of patients died either of the consequences of HSCT or due to malignancy.

ATM was diagnosed in 10 patients, six of whom suffered from severe immunodeficiency and underwent substitutional immunoglobulin therapy. Four ATM patients developed malignancy and three of four died of its consequences.

Six patients were diagnosed with NBS. Five of six patients (83%) suffered from immunodeficiency, substitutional therapy was administered in 83% of patients. One patient showed autoimmunity, same patient was the only one to develop lymphoid

malignancy to this point. All patients were alive. Artemis deficiency was diagnosed in one patient. This patient was diagnosed with Omenn syndrome and radio-sensitive severe combined immunodeficiency (RS-SCID) soon after birth and received curative HSCT early on.

This study delivered estimates on the prevalence of these rare diseases in Austria. Furthermore, it provides data on the clinical course in the context of prophylactic and therapeutic measures such as HSCT, confirmed the heterogeneity of clinical patterns within the syndromes, stressing how crucial timely diagnosis and personalized treatment are for the wellbeing and survival of affected patients.

Zusammenfassung *

** Im Folgenden wird die männliche Form verwendet, soll jedoch für beide Geschlechter repräsentativ sein.*

Chromosomeninstabilitäts-Syndrome mit DNA-Reparaturdefekten bilden eine seltene Grppe von Erkrankungen im Kindesalter, welche (hauptsächlich) autosomal rezessiv vererbt sind. Manifestation und Schweregrad solcher Syndrome können von Patient zu Patient variieren. Häufig sind kongenitale Fehlbildungen vorhanden, so etwa Veränderungen im Skelettsystem, der Haut und des Urogenitaltraktes, Mikrozephalie und andere mehr. Weitere wesentliche Krankheitsmerkmale sind progressives Knochenmarksversagen oder Immundefekte, und Prädisposition für maligne Erkrankungen. Auf molekularer Ebene weisen betroffene Patienten Hypersensitivität auf DNA-quervernetzende Substanzen und starke Strahlensensibilität auf.

Das Ziel dieser Studie war es zu ermitteln, welche der Syndrome in Österreich vertreten sind, wie viele Patienten betroffen sind, wie deren Krankheit voranschreitet und welche Therapien veranlasst wurde.

Dazu wurde eine relationale Datenbank entwickelt. In diese wurde Information von Patienten, die im Jahr 2000 und später am Leben waren und direkt von hämato-onkologischen Abteilungen in Österreich stammt, eingespeist. Diese Datenbank soll als Basis eines zukünftigen österreichischen Registers für Chromosomeninstabilitäts-Syndrome dienen.

Es konnten siebenundvierzig Patienten identifiziert werden, die vier verschiedene Syndrome repräsentieren: Fanconi Anämie (FA), Ataxia teleangiectasia (AT), Nijmegen-breakage Syndrom (NBS) und Artemis Deficiency (AD). Patienten mit anderen Syndromen, wie etwa Bloom Syndrom, Turcot Syndrom (Konstitutives Mismatch-Repair-Syndrom), DNA-Ligase-4-Deficiency oder Cerunnos Deficiency konnten nicht identifiziert werden.

Alle 30 FA-Patienten zeigten progressives Knochenmarksversagen, 70% wurden stammzelltransplantiert. Malignome wurden bei 57% der Patienten beobachtet, unter anderem Myelodysplastisches Syndrom (MDS), akute myeloische Leukämie (AML) und Plattenepithelkarzinome. An den Konsequenzen der Stammzelltransplantation oder auf Grund von Tumorleiden verstarben 33% der Patienten. ATM wurde bei zehn Patienten diagnostiziert, sechs von diesen wurden

auf Grund der Immunschwäche mit Immunglobulin-Ersatztherapie behandelt. Vier Patienten entwickelten Malignome, drei dieser vier Patienten verstarben daran. NBS wurde bei sechs Patienten diagnostiziert. Fünf dieser sechs Patienten litten an Immunschwäche und erhielten Ersatztherapie. Ein Patient zeigte Autoimmunität und war der einzige dieser Gruppe, der ein Malignom entwickelte. Alle Patienten waren am Leben. AD wurde bei einem Patienten diagnostiziert. Dieser Patient wurde kurz nach Geburt mit Omenn-Syndrom und radiosensitivem, schwerem Immundefekt diagnostiziert und erhielt eine Stammzelltransplantation.

Die vorliegende Studie gibt Auskunft über die Prävalenz der beschriebenen seltenen Erkrankungen in Österreich. Die Analyse aller Daten konnte die Heterogenität des klinischen Erscheinungsbildes innerhalb der einzelnen Syndrome bestätigen und betont gleichzeitig, wie wichtig die frühzeitige Diagnose und personalisierte Behandlung für das Wohlsein und Überleben der Patienten sind.

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Abbreviations

AA	Aplastic anemia
a/cGvHD	acute/chronic GvHD
AD	Artemis deficiency
AFP	Alpha-feto protein
AG	Androgen therapy
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ASD	Atrial septal defect
ATG	Anti–thymocyte globulin
ATM	Ataxia telangiectasia
BM	Bone marrow
BMF	Bone marrow failure
BT	Blood transfusion
BE	Base excision
Bu	Busulfane
CY	Cyclophosphamide
DSB	Double strand break
GIT	Gastrointestinal tract
GvHD	Graft vs. host disease
Hb	Hemoglobin
HLA	Human leucocyte antigen
HR	Homologous recombination
HNSCC	Head and neck squamous cell carcinoma
HSCT	Hematopoietic stem cell transplantation
Ig	Immunoglobulin
MDS	Myelodysplastic syndrome
MM	Mismatch repair
MOF	Multi organ failure
NBN	Nibrin
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
PSC	Peripheral stem cells
SD	Matched sibling donor
URD	matched unrelated donor
TBI	Total body irradiation
TLI	Total lymphoid irradiation
TLS	Translesion synthesis
TSH	Thyroid stimulating hormone
SCC	Squamous cell carcinoma
Sero	Serotherapy
VUR	Vesicoureteral reflux
WBC	White blood cells

1 Introduction

Patients with DNA-repair deficiency syndromes represent a major challenge as their clinical presentation and the course of their disease can be extremely variable.
The syndromes represented in Austria are Fanconi Anemia, Ataxia telangiectasia, Nijmegen breakage syndrome and Artemis deficiency, a group of autosomal-recessive disorders, all of which are known to be caused by defects in DNA-repair-mechanisms. Patients affected by these syndromes are all prone to develop malignancies early on in life, immunodeficiency is a major feature of the latter three.
Due to the low frequency of these disorders, handling of the patients relies on consensus guidelines with constant additions based on clinical experience case by case.
In this thesis project patient information of children, adolescents, and young adults affected by hematologically and/or immunologically relevant DNA repair deficiencies in Austria was collected during 2015-2016 (covering a time span from early 1990ies until today), to depict their current management and treatment, analyze possible weaknesses in individual procedures to provide proposals for future improvements in patient care. An anonymized database was created to serve as the base for a future registry for chromosome-instability syndromes to accumulate patient information from prospective clinical observation, provide long term follow-up and other clinical and laboratory data as well as a network of physicians for possible future studies.
Limitations were data accessibility as well as poor patient documentation.

1.1 Molecular pathophysiology – DNA-repair mechanisms and V(D)J recombination

The clinical presentation of Fanconi anemia, Ataxia telangiectasia, Nijmegen breakage syndrome and Artemis deficiency result from defects in DNA-repair mechanisms that resolve double strand breaks (DSB).
DSBs can be caused through ionizing radiation, radiomimetic drugs such as mitomycin, anthracyclines and topoisomerase inhibitors as well as reactive oxygen species, released under stress.[1]

Six repair mechanisms are known: base excision repair (BE), nucleotide excision repair (NER), mismatch repair (MM), translesion DNA synthesis (TLS), homologous recombination (HR) and nonhomologous endjoining (NHEJ), as reviewed by Kennedy and D'Andrea in 2006.[2]

While HR and NHEJ are involved in the repair of DSB in replicating cells, NHEJ is also involved in V(D)J-recombination, a process important for in T- and B-lymphocyte maturation in the immune system.[3]

Defective DNA damage response results in genomic instability, which predisposes to a wide variety of cancers.[4] Given the involvement of NHEJ in immunological processes, it can also lead to immunodeficiency if aberrant.

1.2 Fanconi anemia (FA)

Fanconi anemia is a rare autosomal recessive (and X-linked) childhood disease and was first described in 1927 as a „familiar infantile perniciosa-like anemia" by Guido Fanconi.[5] Its main features are congenital malformations, progressive bone marrow failure, predisposition to malignancy and, on a cellular level, chromosomal instability and hypersensitivity to crosslinking agents.[6–9] It occurs almost equally in males and females with a ratio of 1.2:1.[10]

The carrier frequency has been estimated to 1:181 in North Americans, 1:93 in Israel and less than 1:100 in specific populations such as Ashkenazi Jews, northern Europeans, Spanish gypsies and others.[11] The exact prevalence is not known, however, in these subpopulation it has been estimated 1 case per 30.000-40.000 births.[12,13]

1.2.1 Molecular background, genetics

The clinical manifestation of FA is due to biallelic mutation in genes involved in DNA repair of double strand breaks, through the mechanism of homologous recombination.[1] The defects in DNA-repair mechanism lead to genomic instability, which predisposes to a variety of cancers.

Nineteen Fanconi genes are known, which act as suppressors of interstrand cross-linking agents and build the so called Fanconi anemia pathway. It can be separated in

two complexes. The core complex, consisting of 9 proteins (FANC-A, -B, -C, -E, -F, -G, -L, -M,-T) is believed to be involved in the recognition of the DSB.[14]

This complex activates the heterodimer FANCI-FANCD2, which serves as a scaffold for proteins directly involved in the process of homologous recombination, such as BRCA1, BRCA2 and RAD51, forming a second complex.[15–17] A recent report summarizes the genes and the pathologies associated with their inactivation, known to be involved in the FA pathway.[18] A table extracted from their review can be found in the appendix. (*Table 1*)

1.2.2 Sensitivity to crosslinking agents

As mentioned above, a main characteristic of FA is hypersensitivity to crosslinking agents. This feature is being used in the diagnostics of FA. The chromosomal breakage rate of lymphocytes can be evaluated by supplementing the substances Mytomycin C (MMC) or Diepoxybutane (DEB), and is typically elevated in FA, rearrangements such radial figures can be seen as well.[19]

Negative findings don't exclude the diagnosis of FA. In patients with otherwise typical FA-like features, mosaicism could be present. This can also account for patients with a chromosomal breakage rate higher than found in the general population but much lower than the typical FA elevation.[20,21] Alternatively, a different syndrome present hypersensitivity to MMC or DEB, resulting in high chromosomal breakage. For instance, other syndromes with defective DNA repair mechanisms exist, such as Ataxia telangiectasia (ATM), Ataxia telangiectasia-like disorder, Nijmegen breakage syndrome (NBS), Nijmegen breakage-like syndrome, Bloom syndrome, DNA ligase 4 syndrome, Seckel syndrome, Artemis deficiency, Cernunnos deficieny, Dyskeratosis congenita and some of the severe combined immunodeficiency (SCID) syndromes. But also others like Dubowitz syndrome, Roberts syndrome, Rothmund-Thomson syndrome and Warsaw breakage syndrome show elevated rates of chromosomal breakage. [22–33]

1.2.3 Congenital malformations

A wide range of physical abnormalities have been reported in FA. 50-70% of patients present with abnormalities such as growth retardation, skeletal abnormalities and skin pigmentation irregularities.[6,34,35] Up to 30% of patients present with less frequent or no physical abnormalities at all.[36] *Table 2* offers an overview of all congenital malformations, as summarized in the guidelines of the German Fanconi anemia Registry.[37]

Table 2. Physical abnormalities in Fanconi anemia

Skin	Café-au-lait spots, hyper- and hypopigmentation
Growth	Intrauterine growth retardation, short stature, endocrine abnormalities
Eyes	Microophthalmia, short or almond shaped palpebral fissures, ptosis, epicanthal folds, hyper- and hypotelorism, strabismus, cataracts
Ears	Deafness (usually conductive), abnormal or absent pinna, prominent ears, abnormally positioned ears (lowset or posteriorly rotated), small or absent ear canals, absent tympanic membrane, microtia, fused ossicles
Thumb and radius	Thenar hypoplasia, absence or hypoplasia of radius and/or thumb, floating thumb, bifid thumb, digitalized thumb/abnormal thumb placement
Other skeletal	Dysplastic or absent ulna, micrognathia, frontal bossing, spina bifida, Klippel-Feil, vertebral anomalies, absent clavicles, Sprengel's deformity, Perthes disease, congenital hip dysplasia/dislocation, scoliosis, rib abnormalities, clubfoot, sacral agenesis (hypoplasia), leg length discrepancy, kyphosis, brachydactyly, arachnodactyly, humeral abnormality, craniosynostosis
Kidney and urinary tract	Ectopic, horseshoe, rotated, hypoplastic or absent, dysplastic, hydronephrosis, hydroureter, urethral stenosis, reflux
Genital	Males: micropenis, penile/scrotal fusion, undescended or atrophic or absent testes, hypospadius, chordee, phimosis, azospermia Females: bicornate uterus, aplasia or hypoplasia of vagina and uterus, atresia of vagina, hypoplasic uterus, hypoplastic/absent ovary, hypoplastic/fused labia
Cardio-pulmonary	Patent ductus arteriosis, ventricular septal defect, pulmonic or aortic stenosis, coarcation of the aorta, double aortic arch, cardiomyopathy, tetralogy of Fallot, pulmonary atresia
Gastro-intestinal	Esophageal atresia, duodenal atresia, anal atresia, tracheoesophageal fistula, annular pancreas, intestinal malrotation, intestinal obstruction, duodenalweb, biliary atresia, foregut duplication cyst
Central nervous system (CNS)	Microcephaly, hydrocephalus, Bell's palsy, CNS arterial malformations, abnormal pituitary, absent septum pellucidum/corpus callosum, hyperreflexia, neural tube defection, Arnold-Chiari malformation, Moyamoya, single ventricle

This table was taken form the protocol of the German Fanconi Anemia registry. The content has not been changed, but the layout has been modified.

1.2.4 Hematologic manifestations

1.2.4.1 Progressive bone marrow failure and hematologic malignancies

Progressive bone marrow failure is a severe feature of FA. According to studies, the median age at symptom onset is 7.6 years, however it may vary depending on the underlying mutation. [38,39]

Hematologic manifestations are usually the first serious symptoms leading patients to the hospital. They present themselves with individually severe forms of thrombocytopenia, anemia and leukopenia.[39] Some patients remain stable, whereas in others BMF progresses quickly.

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) can be present at diagnosis or occur over the course of time. The incidence of developing BMF by the age of 40 is 90%[38], the risk of identifying clonal cytogenetic abnormalities at the time of BMF manifestation was reported at 67% by the age of 30. The risk of developing either MDS or AML by the age of 40 is 52%.[39] The median age of leukemia onset has been described at 11.3 years.[40]

1.2.4.2 Treatment options

Several treatment options have been established in order to control haematologic manifestations of FA.

- Blood transfusion
- Androgen therapy
- G-CSF
- Hematopoietic stem cell transplantation (HSCT)

Patients with low count in red blood cells (HB<7) or thrombocytes or symptomatic anemia with bleedings can receive transfusions. Transfusions of blood products are a limiting, merely symptomatic treatment. An adverse influence of red blood cell transfusions on a possible later HSCT has been described.[41]

The use of androgens with substances such as Oxymetholone or Danazol has become a welcome therapy approach.[42] Red blood cells are the most responsive, reticulocytes and Hb increase within the first two months.[10] Platelet counts may take several months to raise[43], neutrophil counts can also improve.[44,45] The required dosage appears to be very individual, some patients may require only very little dosages daily or even

weekly.[43] Other patients seem to develop an androgen resistance over time, needing higher and higher dosages.[10] Side effects appear dose dependent and pose the limiting factor of androgen therapy. Common side effect are: acne, priapism, virilization, growth spurt with subsequent premature closure of growth plates, behavioral changes, liver toxicities such as elevated liver enzymes, cholestasis, peliosis hepatis, liver adenoma/tumors, hypertension, blood lipid changes.[45,46]

Patients with recurring or serious infections and low ANC counts (<500/mm^3) can be treated with G-CSF. If no effect can be seen therapy may be discontinued after two months.[47,48]

Should androgen therapy fail, reach its limits or lead to the development of liver adenoma, or when MDS or AML occur, allogeneic HSCT is indicated.

HSCT comes with many side effects and risks and represents a great challenge for FA patients. Due to the chromosomal instability present in all cells in FA, patients do not tolerate common conditioning regimes. Protocols have been adapted, e.g. by the addition of fludarabine.[49,50] Enhanced immunosuppression can be achieved without added toxicity, thus improving the engraftment.[50,51] Furthermore *in* or *ex vivo* T-cell depletion has significantly decreased the incidence of acute and chronic graft-versus-host disease (GvHD).[52,53]

Studies show survival rates of 70-100% with allogeneic transplantations of matched sibling donors (MSD), transplants with alternate donors (AD; mismatched sibling donor, matched unrelated donor) show survival rates of 50-90%.[53]

1.2.4.3 Graft-versus-host disease

GvHD represents a difficult complication of HSCT in patients with FA and presents a challenge in treatment. Due to the underlying DNA-repair defect patients bear a hypersensitivity to TNFα (tumor necrosis factor α) and INFγ (Interferone-γ), thus reacting with an enhanced immune response to HSCT.[54–57] Additionally, it has been shown in tissues affected by GvHD, that cells after HSCT have an increased tendency to undergo apoptosis after transplantation.[58] Toxicities associated with GvHD medications, such as nephrotoxicity related to calcineurin inhibitors and steroid-induced hyperglycemia are known side effects. Yet another problematic aspect is that GvHD has proven to be a risk factor in the later development of squamous cell

carcinomas, with a cumulative incidence of 20% at 8.3 years and 53% at 10-15 years after transplantation.[59–61]

1.2.5 Predisposition to cancer

The most common solid cancer seen in Fanconi anemia is the squamous cell carcinoma of head and neck (HNSCC) and anogenital region. The typical age at onset is 20-40 years, which, compared to the general population (50-60 years), is much younger. Patients previously transplented tend to develop such tumours even 10 years earlier.[59] FA patients have a 500-700 fold higher risk of developing HNSCCs with a 14% cumulative incidence by the age of 40.[38,62,63] Given the risk of death by a hematologic malignancy with 81% by age 40 and a median survival of 23 years, it is assumed the incidence of solid malignancies would be much higher, had they a longer life expectancy.[39,64] Additionally, FA patients seem to develop second primary cancers more frequently (>60%) than the general population (30%)[65]. Mutations in genes that cause FA increase susceptibility of HPV induced carcinogenesis. Though studies lead to believe that the inactivation of p53 through HPV-associated oncoproteins may be causative rather than by direct mutagenesis.[66]

Since alcohol and tobacco are known risk factors for developing HNSCC in the general population (risk 2-3 fold higher risk with chronic consumption of alcohol, 10-20 fold higher risk with chronic consumption of alcohol and tobacco), consumption should be avoided. Several reports discuss the risk of developing HNSCC due to poor oral hygiene and chronic, repeated trauma, thus maintenance of oral hygiene represents another preventative method. HPV vaccination for all individuals not yet having undergone puberty is recommended, with regular screenings as of the age of ten.[64]

1.2.6 Treatment of (HN)SCC

In Fanconi patients, healthy, non-cancerous cells are highly sensitive to treatments that crosslink DNA (chemotherapeutic cisplatin, radiation). At the same time FA-HNSCC cells in seem to be less sensitive to crosslinking agents, compared to FA-non-cancerous cells. They are typically highly aggressive and often present themselves in advanced stages. Given the poor tolerance to radiation and chemotherapy, surgery remains the only viable option.[67,68]

1.2.7 Non-HNSCC in FA

Case series, case reports and cohort studies have reported the occurrence of other solid malignancies than HNSCC in FA, with a 50-fold higher risk than in the general population.[69] Breast cancer, brain tumors (neuroblastoma), liver adenomas and carcinomas, osteosarcoma, soft-tissue sarcoma, skin squamous cell carcinoma and skin basal cell carcinoma have been reported.[63,69–71]

1.3 Ataxia telangiectasia

Ataxia telangiectasia is an autosomal recessive syndrome, first described by the French physicians Syllaba and Henner in 1926.[72] Its main characteristics are progressive cerebellar ataxia, oculocutaneous telangiectasis, recurrent infection of the respiratory tract, cellular hypersensitivity towards ionizing radiation and predisposition to cancer. [73–76]

The clinical pattern is the result of a mutation in ATM (Ataxia telangiectasia mutated), which is located on chromosome 11q22-23. [77,78]

The frequency of AT is difficult to distinguish, it varies considerably from country to country. In the United States it seems to appear approximately in 1 per 40.000 live births worldwide.[79]

1.3.1 Molecular background

ATM is part of a super-complex, called BASC. Together with other genes, it is involved in the recognition double strand breaks (DSB) and also plays a role in the mechanisms of HR and NHEJ.[80,81] The inability to repair DNA defects sufficiently results in a high risk to develop malignancy, as well as an impaired function of the immune system. Recently, it has also been shown that ATM plays a crucial rule in neurons, mediating the repair of DSBs, just as in proliferating cells.[82]

Furthermore, it has been described, that ATM is abundant in neurological progenitor cells and important for the maintenance of their stability, helping in proper proliferation, survival and differentiation. Moreover, it is reqired for proper terminal differentiation of neural stem cells. Thus, if inactive through mutation, instead of reacting properly to cell damage, resulting in neurodegeneration. [83,84]

1.3.2 Hypersensitivity to ionizing radiation

Patients with AT show hypersensitivity to ionizing radiation and radiomimetic substances (such as actinomycin C, mitomycin C). A high frequency of chromosome breakage as well as characteristic chromosomal aberrations can be seen in *in vitro* testings of lymphocytes. [32,33]

1.3.3 Phenotypic manifestations

AT affected children usually appear normal at birth. The first signs of ataxia begin to show as soon as they start walking and become apparent by the age of 2-3 years with an unsteady gait and staggering. Swaying of the head and trunk on standing and even sitting may appear. The ataxia is progressive and soon their movements become athetoid, lateron dyssynergia and intention tremor of the upper extremities become prominent features. AT patients are hypotonous, their tendon reflexes diminished. Their posture changes and becomes rigid, stooped with dropped shoulders, their head sunk forward and often tilted to one side, their faces mas like, their gait brad based. At the age of ten years the ataxia and weakness have usually progressed to a point that requires the use of a wheelchair. [73,74,79,85,86]

Hair and skin are dry and coarse in AT patients, café au lait spots are frequently found.[73,85]

Telangiectasia has a later onset at about 4-6 years of age. They are first noticed in the conjunctivae as fine horizontal bright red lines and progress to simulate conjunctivitis. The spread symmetrically to the butterfly area of the face, further to the neck and antecubital and popliteal spaces and finally can be found on the dorsum of hands and feet. [73,85,86]

Oculomotor apraxia and dysarthria appear early on but may be difficult to evaluate in younger children. They start talking normally, but characteristically slow. Over time their speech becomes indistinct, slurred and scanning.[73,85]

Statural growth in AT patients slows down progressively, mental retardation is usually not found though IQ scores tend to drop below normal range as ataxia progresses.[73,85]

1.3.4 Immunodeficiency & laboratory findings

Patients with ataxia telangiectasia suffer from recurring infections of the upper and lower respiratory tract. They often present a chronic cough, chronic sinusitis and crackling rales.[85]

The susceptibility to lower respiratory tract infections increases, though the underlying immunodeficiency is not progressive, so the cause may rather be the increasing feebleness, progressive abnormalities of chewing and swallowing, leading to pulmonary aspiration.[87]

Early patient reports describe thymic atrophy in several patients, offering another cause for immunodeficiency.[88–90]

Deficiency of the humoral as well as the cell-mediated immunity is found in AT. The range and severity varies from patient to patient. Typically, prominent reduction in T-lymphocytes are found, with a relative deficiency of total $CD4^+$ cells, a reduced $CD4^+/CD8^+$ ratio and low B-lymphocytes levels.[87,91]

A marked reduction or even absence of IgA and IgG and IgE are common[91], respectively or in combination. Absolute IgG levels may be in the normal range, masking a deficiency of IgG subclasses (mainly IgG2 and IgG4).[92]

Another peculiar finding in AT patients are elevated alpha-fetoprotein levels, the pathogenesis and prognostic relevance of which are not entirely understood.[93–96]

1.3.5 Malignancies in AT

The risk of developing cancer in AT is 61 to 184 times higher compared to the general population.[76] Most malignancies found in affected patients are of lymphoid origin, non-Hodgkin lymphoma, Hodgkin lymphoma, acute lymphoblastic T-cell leukemia and T-cell prolymphocytic leukemia among others. The most common malignancy of non-lymphoid origin is breast cancer. Others are thyroid carcinoma, medulloblastoma, astrocytoma, hepatocellular carcinoma, pancreatic cancer, testicular seminoma, ectopic pituitary tumor, dermatofibrosarcoma protuberans, myeloma, reticulum cell carcinoma and myeloid leukemia.[75,97,98]

The cancer risk of heterozygous relatives has been evaluated in studies and showed an increased risk, estimated as 3.8-fold higher in men and 3.5-fold higher in women. [99–101] The risk for developing breast cancer for heterozygote women is 5.1-fold higher than in the general population. [100,101]

1.3.6 Therapy

No effective therapies for AT are known to this point. Intravenous immunoglobulins (IVIG) are frequently used as a supportive therapy to replace or increase the levels of

circulating antibodies and reduce the frequency of infections. In patients with recurrent bacterial infections prophylactic treatment with antibiotics can be initiated. [87,102]

A new treatment approach for the neurological component of AT is the use of steroids, e.g. bethametasone, which seems to improve the neurological signs, though its use is limited due to side effects.[103,104]

1.4 Nijmegen breakage syndrome

Nijmegen breakage syndrome (NBS) is an autosomal recessive disorder, which was first described by researchers of the University of Nijmegen, Netherlands, in 1981. Progressive microcephaly, mental retardation, immunodeficiency, chromosomal instability and predisposition to malignancy are its main characteristics.[105] Though very similar to Ataxia telangiectasia, it is genetically distinct.[106]

1.4.1 Epidemiology

The exact incidence of Nijmegen breakage syndrome is not known. However, the prevalence of the carrier frequency of the most common mutation has been described as 1 in 866 in the German population. The ethnic origin is eastern europe, manily polish, check and slovakian.[105,107,108]

1.4.2 Molecular background

Mutations in the NBS gene, localized on chromosome 8q21, are responsible for the phenotype.[109,110] The gene codes for the protein Nibrin and belongs to the MRN-complex, together with Rad50 and MRE11.[111,112] This Complex is part of the super-complex BASC and involved in DNA damage response.[81]

1.4.3 Chromosomal instability

Cells of NBS affected individuals show typical rearrangements, preferentially in chromosomes 7 and 14 in the form of inversions and translocations.[113,114]
The cells have been shown to be hypersensitive toward ionizing radiation as well as chemical agents (such as bleomycin).[115]

1.4.4 Clinical phenotype

Patients with the Nijmegen breakage syndrome are microcephalic. Though some patients may present themselves with a normal head circumference at birth, the microcephaly is progressive. Their facies are “bird-like” and become more characteristic with age. A receding forehead, prominent midface, long nose and

philtrum, receding mandible, upward slanting palpebral fissures, large ears with dysplastic helices and freckles on cheeks and nose are typically seen. They are short in stature, proportionate in weight and height.[114,116,117]

Café au lait spots and vitiligo are often found on their skin, clinodactyly, syndactyly or polydactyly have been described in several cases.
Other abnormalities comprise hydronephrosis, ectopic kidneys, lack of secondary sex characteristics, hypospadias in males and ovarian dysgenesis in females, anal atresia or stenosis.[114,117]
The mental development of NBS patients has been described as normal in 35-40%, borderline to mildly retarded in 45-50% and moderately retarded in 10-20%.[114,116,117]

1.4.5 Immunodeficiency

Patients with NBS suffer from recurrent infections of the upper and lower respiratory tract, the urinary tract, and in some cases also gastrointestinal infections have been described. Severe immunologic complications and opportunistic infections are rarely seen.[114,116]
Deficiencies of the cell mediated as well as the humoral compartment are found in NBS.[114,118–120]
Most patients present with reduced absolute numbers of $CD3^+$ T-cells, $CD4^+$ T-cells and $CD8^+$ T-cells, as well as a decreased $CD4^+/CD8^+$ ratio. NK-cells have been described to be elevated or normal, B-cells are reduced in most cases.[114,118,121,122]
The humoral deficiency is apparent mainly in IgG and IgA, isolated IgG deficiency and combinations of all 3 types have been reported.[118,119,123] IgG2 and IgG4 levels, combined or isolated, are frequently diminished.[114,118,123]
IgM levels have been reported to be normal or even elevated in some cases.[118,123] Deficiency in IgM occurs rarely.[114]

1.4.6 Malignancy in NBS

The most common malignancies are Lymphoma (mainly of B-cell type), but other malignancies as precursor T-cell lymphoblastic lymphoma/leukemia, Glioma, Medulloblastoma and Rhabdomyosarcoma have been described [114,116]

A correlation between the sudden and systemic elevation of IgM and the development of lymphomas has been described by Gregorek *et al.* in two studies.[118,124]

1.4.7 Therapy

A specific therapy for NBS does not exist to this date. Depending on the gravity of the immunodeficiency – frequency and severity of recurrent infections, immunoglobulin substitution can be started. In patients with severe immunodeficiency or malignancies resistant to other therapies, HSCT has been carried out successfully.[125]

1.5 Artemis deficiency

Artemis deficiency is an autosomal recessive disorder, which was first described in 1998 as a subgroup of severe combined immunodeficiency with radiosensitivity.[126–128]

1.5.1 Molecular background

The disease causing mutation lies in the Artemis gene (DCLRE1C), located on chromosome 10p.[129,130] Patients with null, as well as hypomorphic mutations have been observed. A complete lack of protein function due to a null mutation abrogates T- and B-cell development with the result of immunodeficiency, susceptibility to autoimmunity, lymphoproliferation and malignancy. Hypomorphic mutations with residual protein function cause a milder phenotype (called leaky SCID).[131]

1.5.2 Clinical features

The most common haematologic features comprise autoimmune hemolytic anemia, immune neutropenia and thrombopenia. The presentation of immunodeficiency is very heterogenous. Recurrent upper and lower respiratory tract infections, urinary tract infections, gastroenteritis, candidiasis infections (oral, intestinal, genital), lymphadenopathy and verruca vulgaris have been described.[126–128,132–137] Soft tissue infections and granulomatous inflammations of the skin and recurrent oral ulcers have been reported in one patient, respectively. Erythrodermatitis, mycobacterial skin infection, Hashimoto thyroiditis, Hepatomsplenomegaly, progressive liver disease and inflammatory bowel disease were also found in different patients.[127,128,132,134–137] Many have failure to thrive. One patient presented with Omenn Abnormality and one with Evans Syndrome.[127,134,135,137]

1.5.3 Immunodeficiency

Lymphopenia is present in all patients, but of different composition. Most patients present with very low[126,127] or absent[132,134,135] CD19 Lymphocytes and very low to

moderately reduced $CD8^+$ and $CD\ 4^+$ cells (respectively[133] or combined[126–128,134,136]). In some patients a progressive loss of $CD4^+$ and $CD8^+$ has been described.[132] Immunoglobulin levels may be normal[126–128] but low IgG [132–134,136], IgA (in some patients even absent [132,134]) levels are seen. IgM levels have been described to vary from absent to normal [132–134,136] to even raised levels of IgM[134]. In some patients immunoglobulin levels may initially be normal but with a progressive decrease [128]. Specific antibody production is impaired in most of the patients. [133,134].

1.5.4 Malignancy

Patients have been described to develop lymphatic tumors. Hodgkin's lymphoma as well as EBV associated lymphomas and large granular lymphocytic T-cell leukemia have been described in several patients.[126,134,137] Carcinoma in situ of the nipple with subsequent bilateral recurrence has been described in one patient.[126]

1.5.5 Therapy

The therapeutic posibilities in Artemis deficiency are limited. Either HSCT or gene therapy are indicated early on, otherwise the rapid course of SCID usually ends letal within first year of life. [138]

2 Research Question and Hypothesis

Fanconi anemia and other hematologically and / or immunologically relevant tumor predisposing syndromes with chromosomal instability and DNA-repair defects are rare inherited childhood syndromes. Manifestation and course of disease are extremely variable within syndromes and both prophylactic management as well as therapy can be challenging and difficult. In Austria, patient numbers of 20 to 30 individuals affected by Fanconi anemia, and smaller numbers of other syndromes were anticipated. Sex ratio was assumed to be equal and patients to be represented in all age groups. Frequent comorbidities were expected to be observed; depending on the type of underlying syndrome, physical abnormalities, abnormalities and malignancies of the hematopoietic system, such as bone marrow failure, myelodysplastic syndrome, acute myeloid leukemia, immunodeficiency, lymphoid malignancies and solid tumors.

In order to provide a nation-wide overview of current diagnostic and management standards, a cross-sectional and retrospective analysis of these syndromes was performed to analyze frequency, diagnostic delay, clinical symptoms, various treatments, and survival probability of children and adolescents with these rare diseases.

Extending these aims, data obtained during the time course of the thesis project allowed further analyses: i), gross *epidemiological* overview of hematologically and immunologically relevant DNA repair defects in Austria; ii) indications, modalities, cytogenetic background, and outcome of HSCT in patients with Fanconi anemia in Austria (including and extending findings of a previous diploma thesis project at St. Anna children's hospital); iii), frequency of severe complications / disease evolution such as incidence and outcome of malignancies in patients with DNA repair defects and immunodeficiencies.

3 Patients and Methods

3.1 Patient recruitment, inclusion criteria, data gathering, & ethics votum

This study included 47 patients with the following inclusion criteria:

- diagnosed with a hematologically and/or immunologically relevant DNA-repair-mechanism
- alive after the year of 2000
- signed informed consent

The recruitment of patients was initiated upon a study proposal and presentation within the Austrian working group for pediatric hematology-oncology (AGPHO) of the Austrian Society of Pediatrics and Adolescent Medicine (ÖGKJ) in March 2014 and a subsequent announcement within the Newsletter of the ÖGKJ[139,140], an internal review of diagnoses represented within the national sub-registry of patients with primary immunodeficiencies (see below), as well as personal communication. In addition, the study proposal and interim analyses were presented at the annual meeting of the ÖGKJ.

Data collection was initiated and performed directly from patient records in pediatric hematology-oncology units of children's hospitals throughout Austria, following the rules of the respective hospitals regarding informed patient consent and good clinical practice. Patients with immunodeficiencies were partially already included in the registry of the European Society of Immunodeficiencies (ESID), thus their data could be obtained from the ESID registry.

For patients with immunodeficiencies that were already registered in the ESID-registry, an informed consent was already existent. Patients diagnosed with Fanconi Anemia were included in the new registry for DNA-instability-syndromes and asked to sign the respective informed consent. The ethics committee of the Medical University of Graz (Institutional Review Board Registry number IRB00002556) reviewed all procedures and gave their approval (EK 27-196 ex 14/15, and yearly consecutive approvals). Patients were anonymized and assigned patient IDs, the key kept separately stored

3.2 Database

The program FileMaker® (*FileMaker Pro Advanced 11.0v2*) was used to create a relational database to store all patient information in an anonymized manner.
The following provides an overview of the relationships within the database and how the data were stored.

3.2.1 Database Infrastructure

To facilitate data transferability of patients with Fanconi anemia, the design of patient record forms and data acquisition for the present study was harmonized with the German registry study FAR01 (coordinated by C. Kratz and H. Hanenberg, Hannover and Düsseldorf, Germany). Patient information was split up in different topics like *main information*, *birth & consanguinity*, *physical abnormalities*, *lab testing*, *malignancies*, *therapy* and *hematopoietic stem cell transplantation & graft vs. host disease.* Each group acted as a separate table, containing detailed information (*Figure 1*).
The patient ID was attributed as primary key to allow to link the tables to each other. The table *main information* serves as the global point of connection for all other tables.

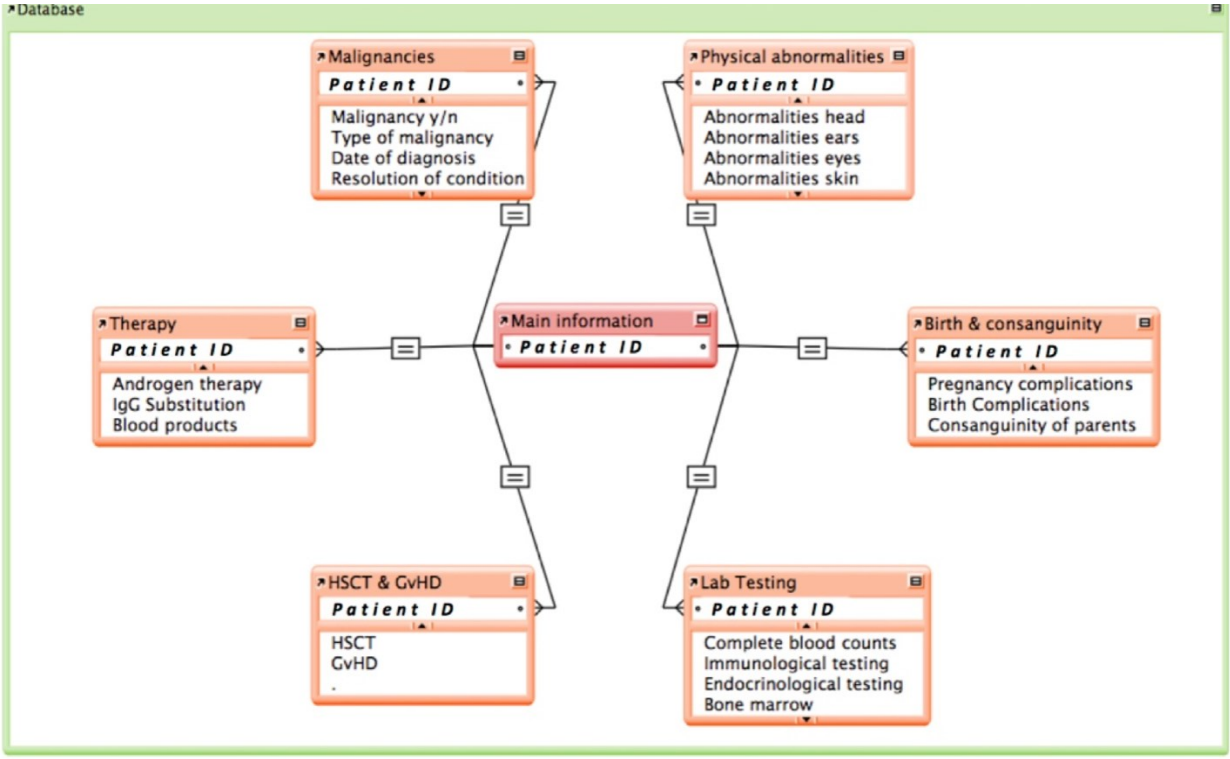


Figure 1. Backbone of the database – schematic display. *The table "main information" serves as a link for the surrounding tables.*

GvHD – graft vs. host disease, HSCT – hematopoietic stem cell transplantation, IgG – immunoglobulin G, n – no, y – yes

3.2.2 User interface

A user friendly interface was created to facilitate data input and visualization of the results. Due to the big amount of information, tables were split up in different categories (referred to as tabs) and are shown in *Figure 1*.

The tab “main info” offers an overview of the most important patient information, as shown in the tab “overview” (*Figure 2a*). Tick-boxes allow to enter information such as gender and survival status. A drop-down menu permits to enter the underlying disease; the dates can be entered either manually or with the help of a drop-down calendar, by pressing the symbol in the right corner. Several editing fields allow the entry of further required information.

The tab “Birth” contains editing fields and tick boxes to enter required information about birth and pregnancy, as well as tick boxes and editing fields regarding family history. (*see Figure 2b)*

Physical abnormalities can be entered in the tab “Physical examination”, numerous check boxes offering “common” abnormalities, as well as comment sections in case the right option is not provided. (*see Figure 2c)*

Laboratory findings are subdivided into further categories e.g. complete blood counts, immunological testing, endocrine testing and bone marrow examinations (*Figure 2d*). A “new entry”-button allows to create several entries at different time points for one patient. Additionally, all previously entered blood counts are depicted in figures, to facilitate long-term monitoring (see *Figure 2d).*

The occurrence of neoplasms is entered in the tab “Neoplasms”, again with a “new entry”-button providing the option of adding malignancies at more than time. A drop down menu offers types of neoplasms, however, information can be inserted manually as well.

Therapies are subdivided into several tabs: androgen therapy, blood transfusions and IgG (immunoglobulin) substitution. *Figure 2e* shows the window for androgen therapy. The “new entry”-box allows to create new records at different time points. This option may be needed in case an adaptation of dosage is undertaken. The substance can be chosen via tick-boxes or, if the required option is not provided, added manually in an editing box. Side effects and stop reasons can be chosen via check boxes, another editing box allowing to add further comments. An additional figure shows the course of the androgen dose regime over time. The last tab “HSCT & GVHD” is subdivided in

tabs for “Stem cell therapy” and “Graft versus host disease” and allow an exact documentation. Information regarding indication, donor relation, type of transplant, conditioning regime and many more viable facts for HSCT can be entered in the first one. The latter one offers tick boxes and editing boxes to determine the degree and type of GvHD.

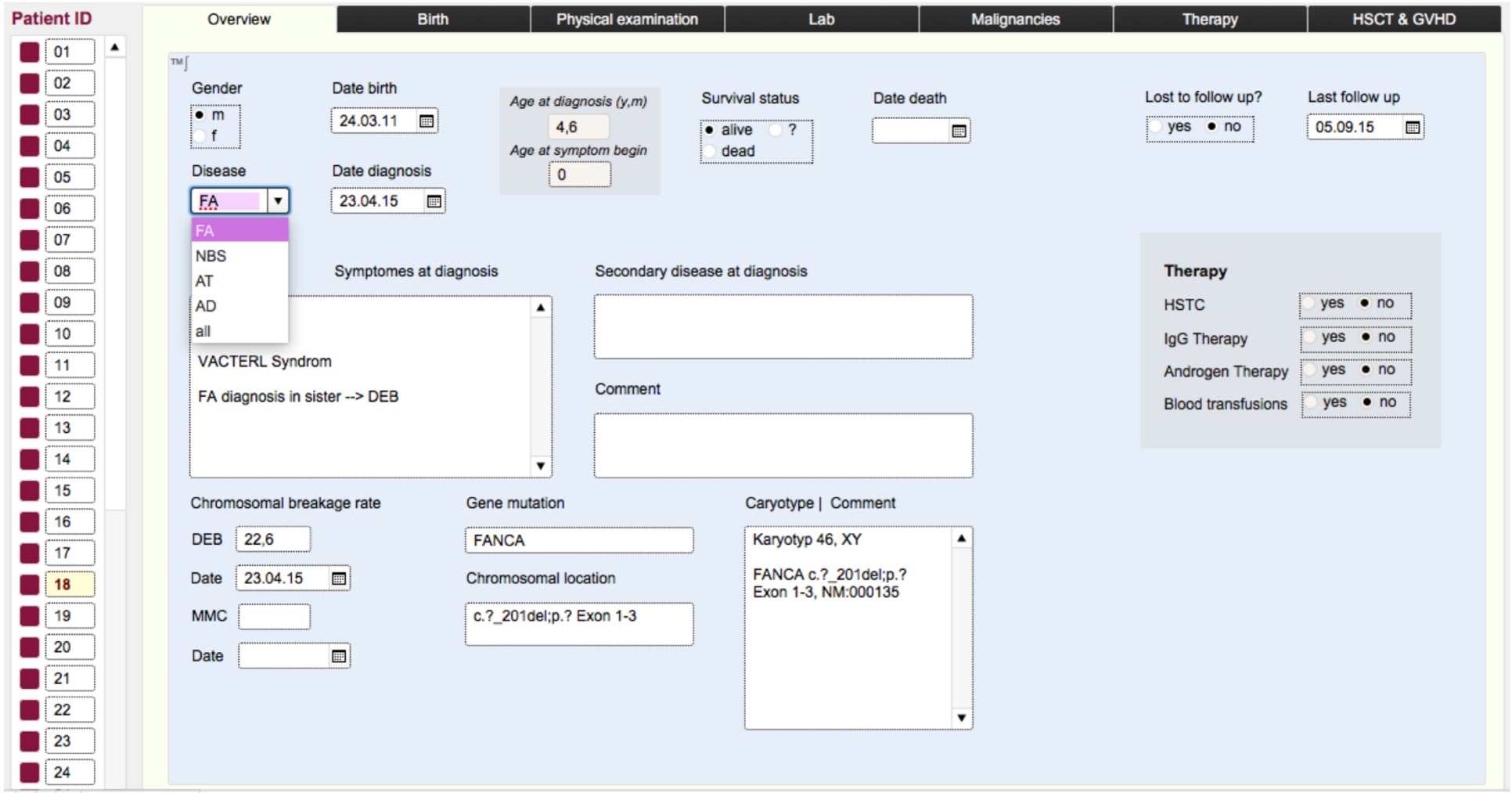


Figure 2a. Overview of most important patient information. *The patient can be chosen by clicking on the red icon next to the patient ID. Patient information can be entered using drop down menus, tick boxes and editing fields.*

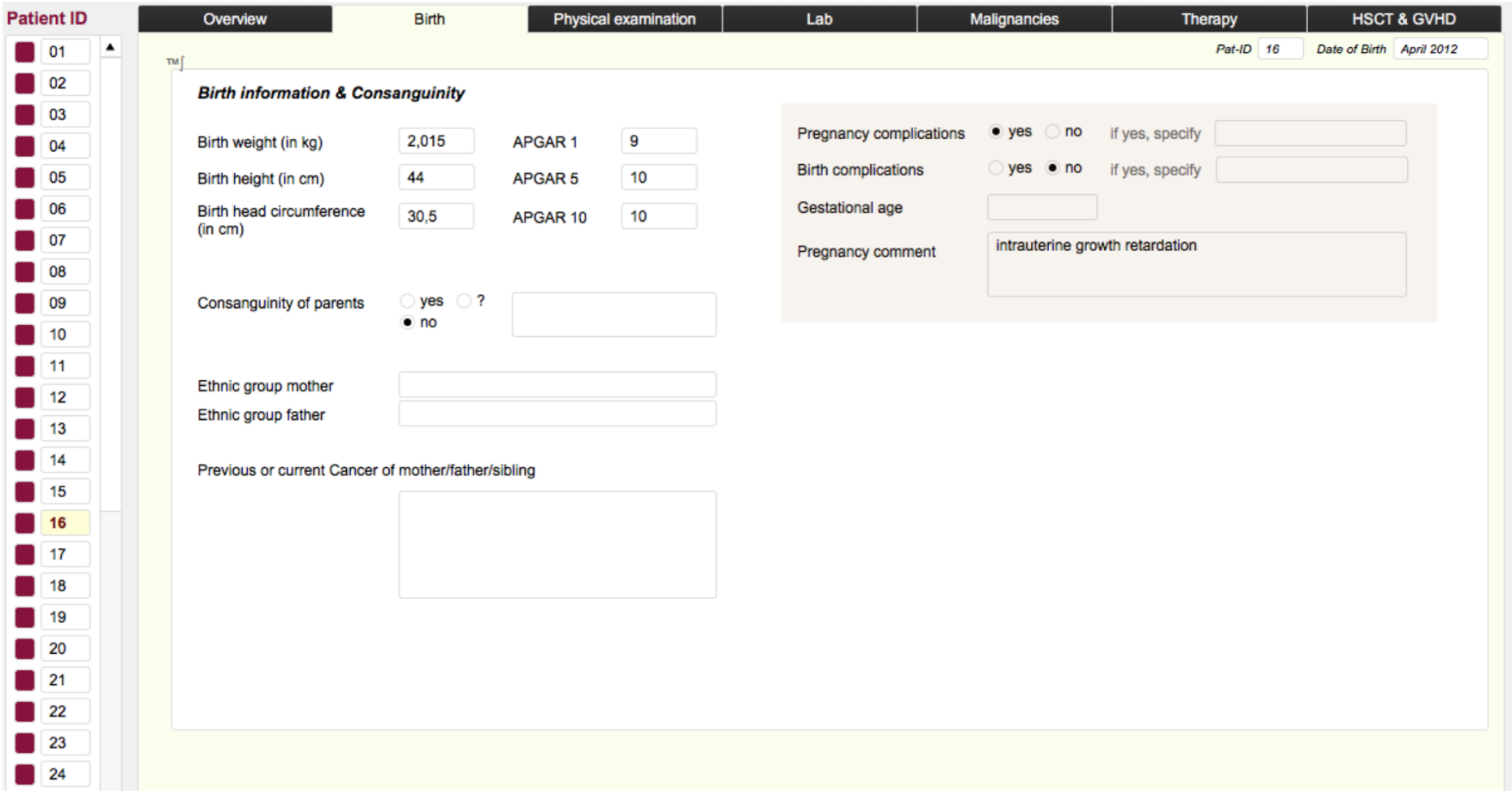


Figure 2b. Entry of birth information and family background. *The patient can be chosen by clicking on the red icon next to the patient ID. Patient information regarding birth, complications during pregnancy/birth as well as family background can be entered using drop down menus, tick boxes and editing fields.*

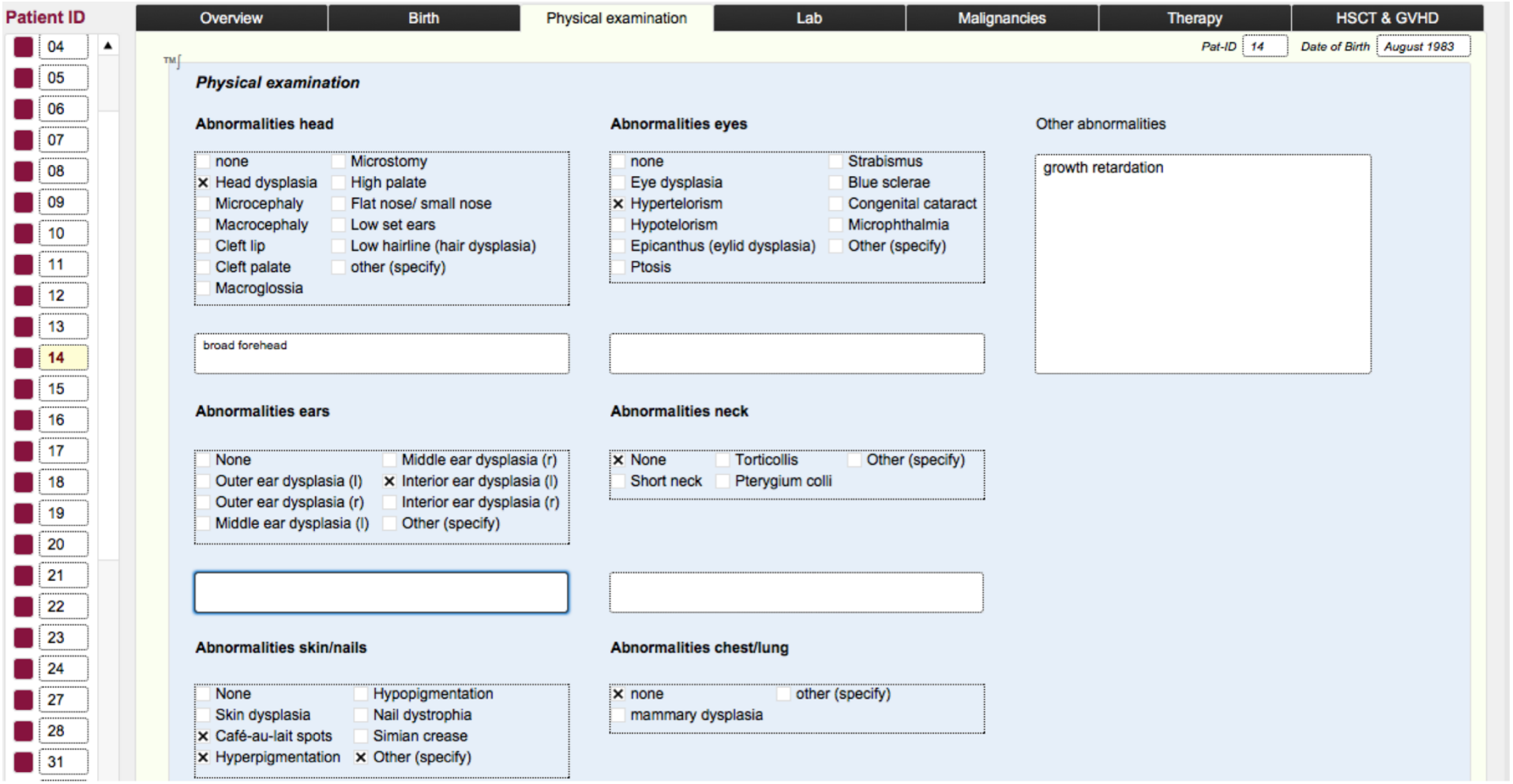


Figure 2c. Physical abnormalities. *The patient can be chosen by clicking on the red icon next to the patient ID. Patient information regarding physical abnormalities can be chosen by using tick boxes or commenting in editing fields..*

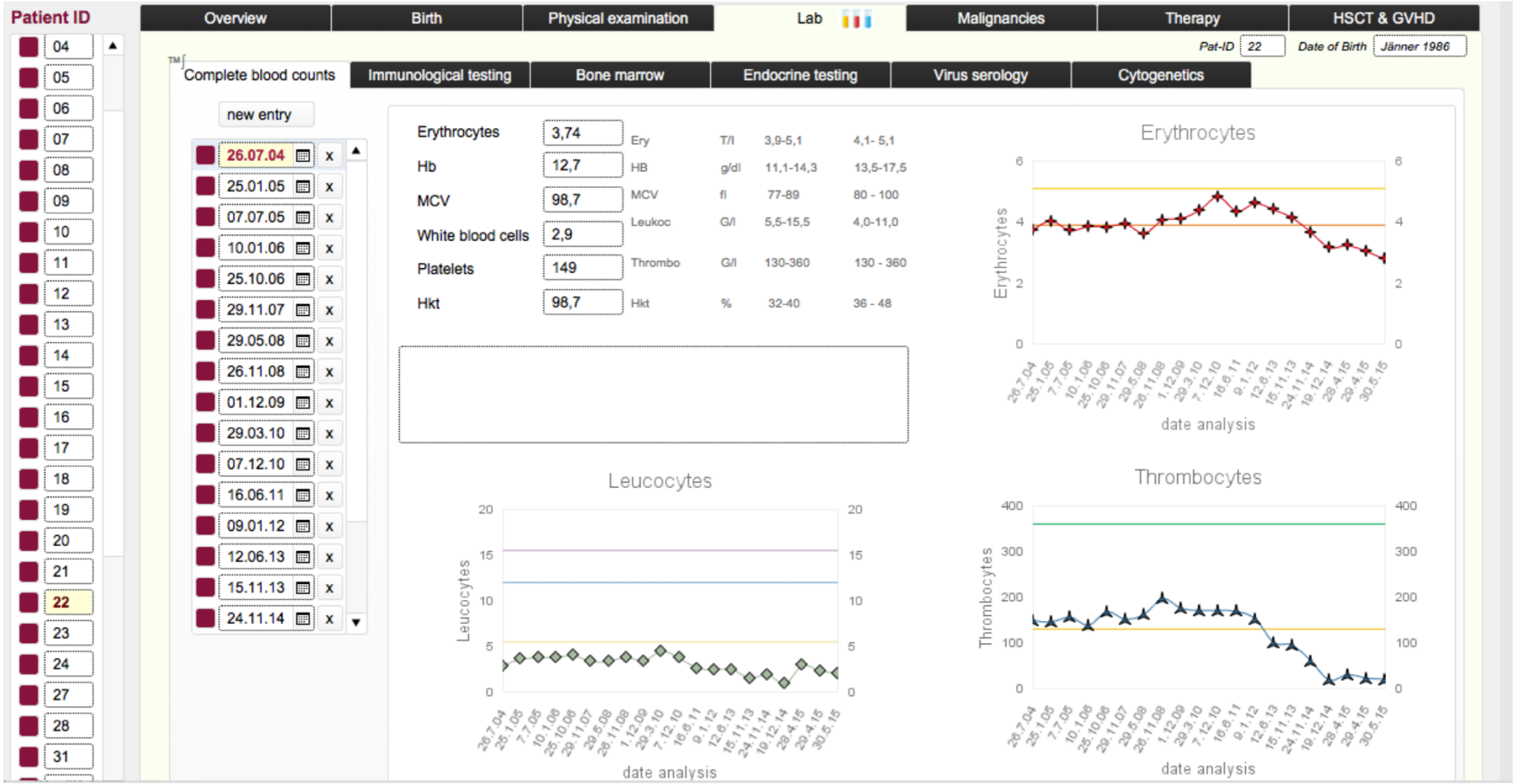


Figure 2d. Entry of blood counts. *The patient can be chosen by clicking on the red icon next to the patient ID. A new form for each count at a specific date can be created by clicking "new entry". Data can be entered in editing fields. Figures "Erythrocytes", "Leucocytes" and "Thrombocytes" show the course over time.*

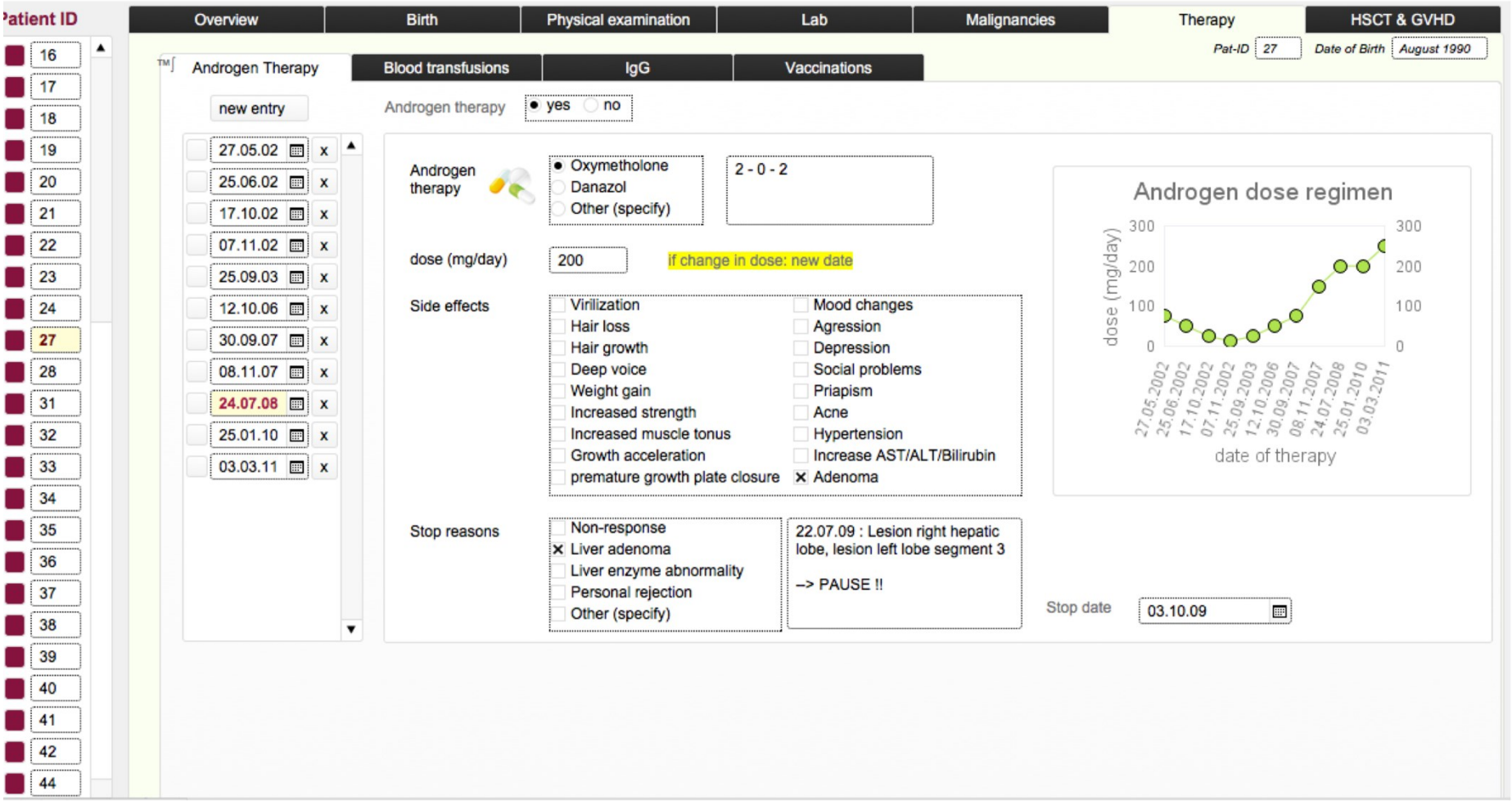


Figure 2e. Androgen Therapy. *The patient can be chosen by clicking on the red icon next to the patient ID. A new form for each change of dosage can be created at a specific date by clicking "new entry". Data can be entered in editing fields, substances can be chosen. Frequent side effects and stop reasons are listed and can be chosen by clicking tick boxes. A figure shows the course of therapy (dosage) over time.*

3.3 Statistical analysis

The Kaplan-Meier method was used to estimate overall survival probability of transplanted Fanconi anemia (FA) patients, divided in groups of younger vs. older than 10 years old. Furthermore, probability of survival in transplanted FA patients was analyzed according to donor type. Statistical significance was calculated using Log-rank (Mantel-Cox) test.

Univariate analyses were conducted to evaluate the impact of key variables in hematopoietic stem cell therapy (HSCT), on the incidence of complications such as graft vs. host disease (GvHD) and graft failure. These variables included two age groups (<10 vs. ≥10 years old) and donor type (sibling donor vs. unrelated donor). Fisher's exact test was used to compare the significance of independence between categorical variables. The statistical analyses were performed with GraphPad Prism®.

A trellis plot was created using TICBO-Spotfire® version 6.4 to depict the course of events in FA patients. The same program was used to show the correlation of age at symptom onset vs. time to HSCT.

4 Results

4.1 Epidemiological data

The following chapter presents the results, first a summary of all and then of the specific syndromes individually.

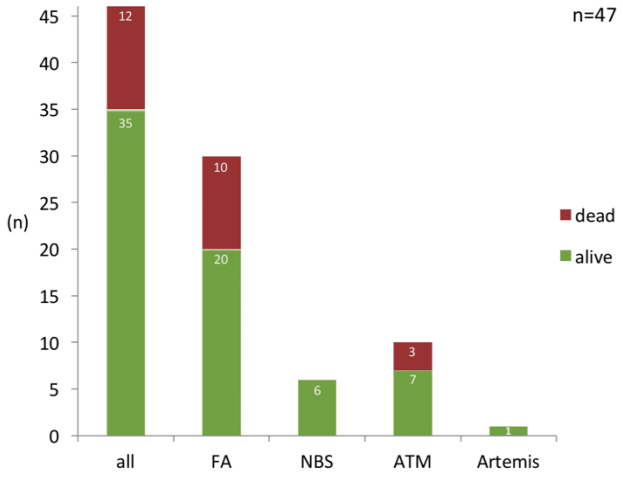


Figure 3. *Survival status of the included patients (last follow up). Patient numbers in columns.*
ATM – Ataxia telangiectasia, Artemis – Artemis deficiency, FA – Fanconi anemia, NBS – Nijmegen breakage syndrome

All patients with a DNA-repair defect that were alive at the year of 2000 and later were included in this thesis. Patient information could be obtained of 47 individuals, provided by several treating institutions throughout Austria. Fanconi anemia (FA) represented the biggest group of patients, followed by Ataxia telangiectasia (ATM), Nijmegen breakage syndrome (NBS) and Artemis deficiency. Survival status was evaluated by analyzing patient records retrospectively. Twenty out of 30 FA patients were found to be still alive at last medical follow-up (FU). The median FU-time of survivors was 7.1 years (range, 0.4-31.4 years). The group of ATM patients included three deceased patients out of 10. The surviving ATM patients showed a median FU-time of 3.8 years (range, 1.4-7.1 years) Six out of six NBS patients as well as the only Artemis patient were alive at the last follow-up. The median FU-time of NBS patients was 7.15 years, (range 0.1-10.8 years), FU of the Artemis patient was 8.1 years. The distribution of patient survival is shown in *Figure 3*. Patients treated in hospitals in Vienna, Graz, Linz, Innsbruck, Salzburg and Klagenfurt were included. Nearly 50% of all patients were treated in Vienna at *St. Anna Children's Hospital* (22 patients) and *AKH Vienna* (1 patient). The second largest group of patients were treated at the *University Hospital of Graz* (12 patients). Five patients were treated in the *Kepler University Hospital of Linz*, four patients in the *University Hospital of Innsbruck*. The *Clinic of Klagenfurt* and the *University Clinic Salzburg* contributed with one NBS and one FA patient, respectively. (*see Figure 4)*.

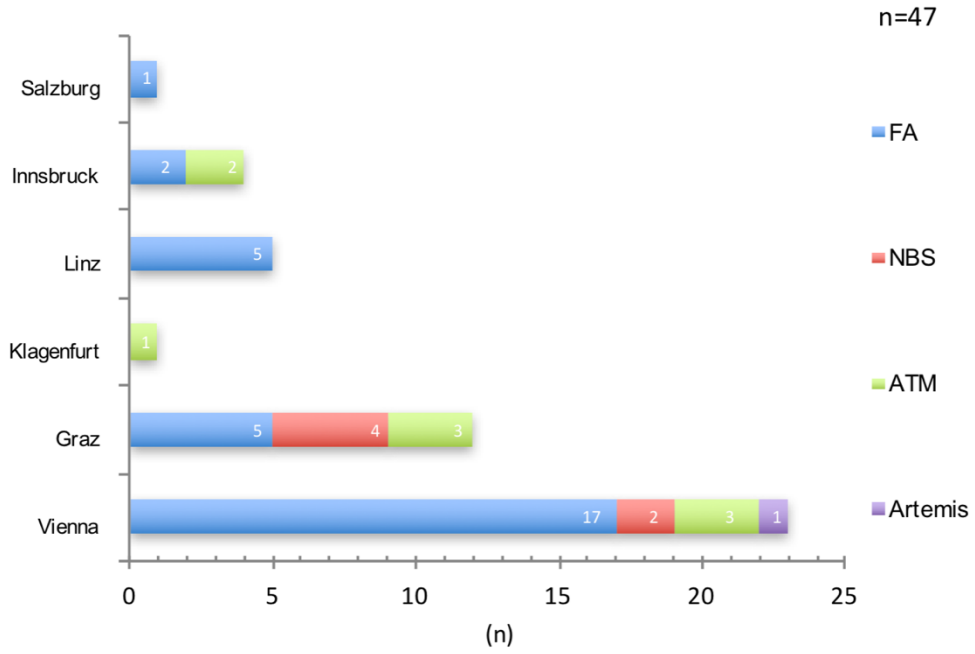


Figure 4. *Distribution of patients and disease subtypes in Austria.*
ATM – Ataxia telangiectasia, Artemis – Artemis deficiency, FA – Fanconi anemia, NBS – Nijmegen breakage syndrome

4.2 Patient characteristics

The main patient characteristics are shown in *Table 3*. Sex ratio, median age at diagnosis, diagnostic delay (time between onset of symptoms and diagnosis), presentation of physical abnormalities and occurrence of malignancies are described. Diagnostic delay was calculated from the time since occurrence of first symptoms (hematological symptoms in FA, neurological or immunological symptoms in ATM, immunological symptoms in NBS and AD) until time of definite diagnosis. diepoxybutane- or mytomycin C-testing was used to confirm FA, whereas, results of genetic testing by identifying mutations associated to NBS, ATM and AD were used to confirm diagnosis. Malignancies were found in 12 FA patients, four ATM patients and one NBS patient.

Table 3. Patient characteristics.

Syndrome	FA	ATM	NBS	AD
Patients (n)	30	10	6	1
Alive|deceased (n)	20|10	7|3	6|0	1|-
Sex f|m (n)	15|15	8|2	2|4	1|-
Age diagnosis (median, years)	6,5	7,7	8,6	-
[range]	[0,1-16,7]	[1,4-18,8]	[0-25]	
Diagnostic delay median (median, years)	0,9*	5,6	8,6	-
[range]	[0-12,7]	[0,4-17,5]	[0-25]	
Physical abnormality present in (n)	26/28*	5/7**	6/6	1/1
Occurence of malignancies in (n) of patients	11	4	1	0

Current status of all included patient groups.

ATM – Ataxia telangiectasia, Artemis – Artemis deficiency, FA – Fanconi anemia, HSCT – Hematopoietic stem cell transplantation, NBS – Nijmegen breakage syndrome

* *only 28 out of 30 patients were evaluable*

** *only 7 out of 10 patients were evaluable.*

4.3 Fanconi anemia (FA)

The sex ratio of 30 FA patients was equal, with 15 males and 15 females. The median age at diagnosis was 6.5 years (range, 0.1-16.7 years) with a median diagnostic delay of 0.9 years (range, 0-12.7 years). Results from genetic testing could be obtained in three patients. Two patients showed a mutation of FANCA, one of them had an additional mutation in FANCI. One patient had a mutation in FANCD1.

Information regarding physical abnormalities was available in 28 out of 30 patients. Over 85% of patients presented physical abnormalities (26 of 28 patients, *Table 3, Figure 5)*.

Figure 6 shows the occurrence of physical abnormalities at different body sites. Microcephaly, flat nose, low set ears and high palate were commonly found within the abnormalities of the head, present in 50% of patients (n=13). Ptosis, epicanthal folds, microphthalmia, hypo- and hypertelorism as well as strabism were abnormalities of the eyes and found in 42%(n=11). Outer and inner ear dysplasia, resulting in hypakusis were present in 27% (n=7) as abnormalities of the ear. Only 11.5% of patients (n=3) presented with abnormalities of the neck, such as pterygium colli, torticollis and short neck. 58% of patients (n=15) showed abnormalities on skin and nails, such as café-au-lait spots, hyper- and hypopigmentation, vitiligo and nail dystrophy. 15.3% (n=4) showed abnormalities of the heart, including right hypertrophic heart, atrial septal defect and valvular heart disease. Common abnormalities of extremities were aplasia of thumb and metacarpal bones, dysplasia and hypoplasia of thumb, thenar and hypothenar, clinodactyly, hexodactyly, radial aplasia and foot dysplasia and were found in 46% of patients (n=12). Skeletal abnormalities in 23% (n=6) included hip and sacral dysplasia, rib aplasia, scoliosis and genua valga. Urogenital abnormalities were found in 65% of patients (n=17), thus the biggest group of abnormalities, and consisted of dystopic kidneys (pelvic kidney, horseshoe kidney, crossed renal ectopy), hypoplastic and agenetic kidneys, hydronephrosis, hypogonadism, phimosis and retractile testes. Gastrointestinal abnormalities were found in 27% (n=7), including rectal, anal, duodenal and esophageal atresia, as well as esophagotracheal fistula. Other abnormalities, such as short stature, were present in 58% (n=15). The median occurrence of physical abnormalities per patient was 5.5 (range, 0-15 abnormalities). Detailed information for each patient can be found in the appendix, *Table 9.*

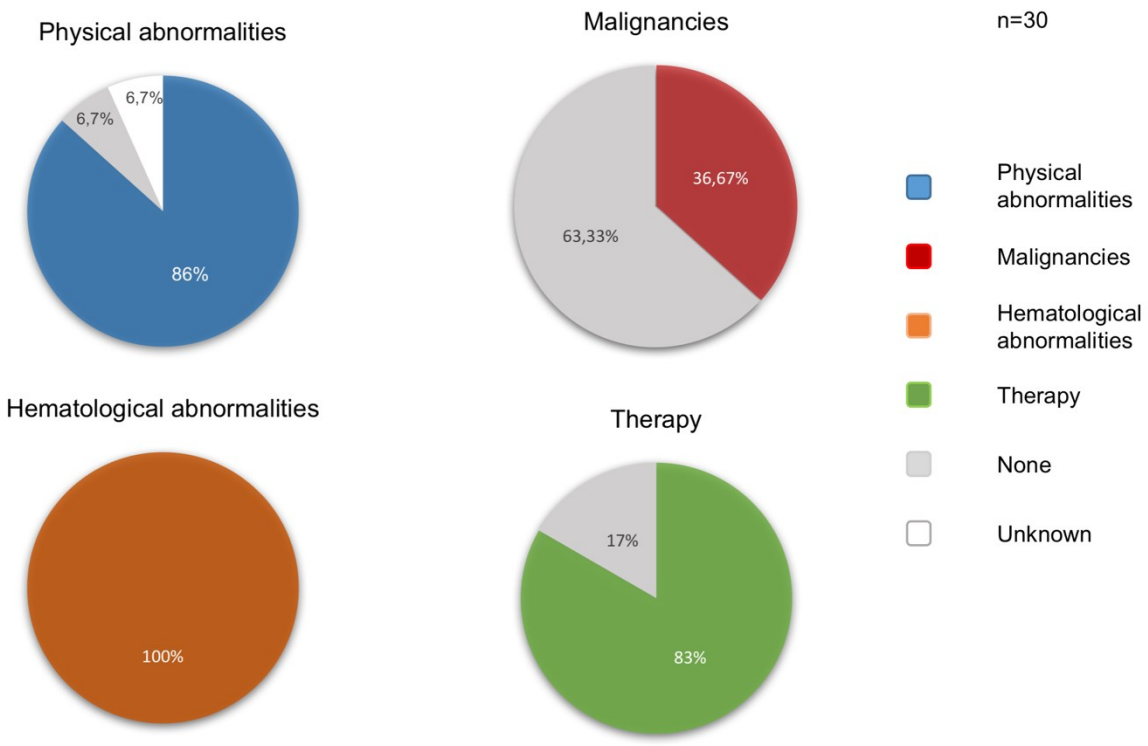


Figure 5. Fanconi anemia: Main characteristics. *Occurrence of physical and hematological abnormalities, malignancies and percentage of patients who required therapy.*

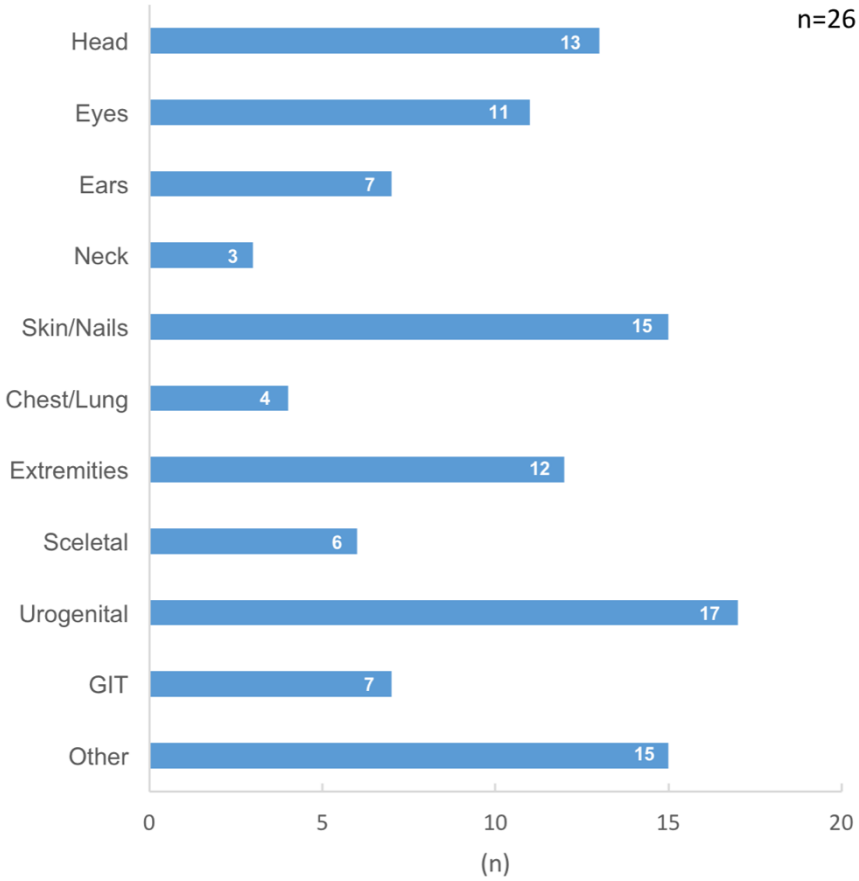


Figure 6. Fanconi anemia: Physical abnormalities. *Occurrence of abnormalities in all patients at different body sites.*

GIT – gastrointestinal tract

Hematological abnormalities were present in all patients (*Figure 5).*
In order to evaluate weather patients had developed bone marrow failure (BMF) and, if so, the degree of BMF; absolute neutrophil counts (ANC), hemoglobin (Hb) and platelet counts were analyzed after diagnosis (see *Table 4*). Only 28 of 30 patients were evaluable, in two patients no sufficient information could be obtained. Analysis over a longer period could not be carried out due to lack of data.
ANC counts were calculated by using the following formula:

$$(\%\ \text{neutrophils} + \%\ \text{bands}) \times \text{WBC} \times 10 = \text{ANC/mm}^3$$

WBC: white blood cells

The analysis of bone marrow failure showed heterogeneous results. Mild, moderate and severe forms of bone marrow failure were represented (*Table 4*).
To evaluate weather patients' blood counts were below the norm ranges, erythrocyte, Hb, platelet and WBC counts were followed since diagnosis until HSCT or last medical follow-up. Two groups were formed: not transplanted and transplanted patients. Given that the norm ranges for blood counts vary at different ages, the minimum range was used as reference to calculate the deviation for each patient considering age and gender. Values are displayed in months since diagnosis.
The group of not transplanted patients consisted of 9 individuals, blood counts were taken at the time of diagnosis until last medical follow-up (*Figure 7a*). In patient 26 data was only available at diagnosis. Patient 16 and 43 were treated with androgens, therapy started 22 or 33 months after diagnosis respectively. Both had a history of blood transfusions. Furthermore, patient 19 received blood transfusions and patient 23 was treated with androgens alone (starting 24 months after diagnosis). The other patients received no therapy.
The majority of FA patients (21 out of 30) received HSCT. In this group blood counts were analyzed from diagnosis until HSTC (*Figure 7b*). During this period patients 9, 11, 27, 29 and 30 received androgen therapy. Androgens were started four months after diagnosis for patient 29, 28 months after diagnosis for patient 27 and 90 months after diagnosis for patient 30. In patients 9 and 11 androgen therapy was started in the same year of diagnosis, although exact dates could not be evaluated. Most patients received blood transfusions, except for patients 6, 13, 22, 24 and 29. The last blood count depicted for each patient was either the last count before transplantation or the

last count available before transplantation. In patients 1, 8, 24 and 25 blood counts were available only at a single time point after diagnosis.

Table 4. Fanconi anemia: Severity of BMF n=28

Patient-ID	ANC/mm^3	Platelets G/l	Hb g/l
P1	703	17	8,4
P2	920	112	7,5
P3	168,3	16	7,9
P4		30	10
P5	2735	37	8,9
P6	930	114	11,7
P7	41,6	19	6,2
P8	466,9	15	9,7
P9	155,1	30	7,6
P10	337,9	41	8,1
P11		32	6,9
P12		48	9,8
P13		32	10
P14		24	10
P15	3831	6	7,1
P16		165	13
P17	2545	122	12,5
P18		31	8,9
P19		12	7,6
P20		64	11,8
P22	1218	149	12,7
P23	1675	68	12
P24	3773	136	10,8
P27		37	8,8
P28		61	12,2
P29	870	29	8,5
P30		41	11,2
P43	1136	51	9,5

within normal range

Criteria	mild	moderate	severe
ANC /mm^3	1000-1500	500-1000	<500
Platelets G/l	50-150	30-50	<30
Hb g/l	≥8 *	<8	<8

Bone marrow failure at one time point after diagnosis. Severity of BMF is color coded.

* *Less than normal for age but > 8 g/dL.*

ANC – absolute neutrophil count, BMF – bone marrow failure
Hb – hemogloin

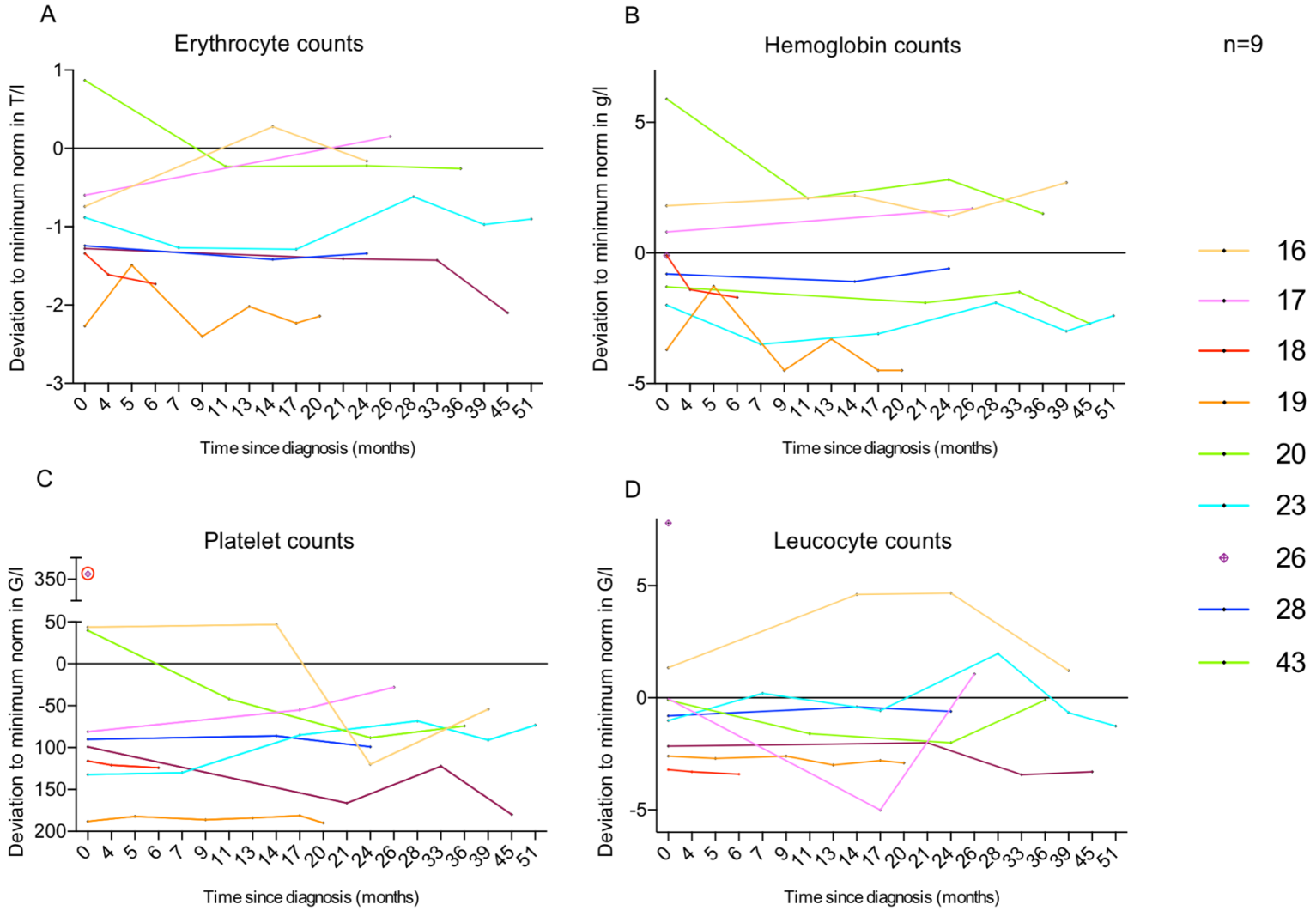


Figure 7a. Fanconi anemia: Blood counts over time in the group of not transplanted patients. *The deviation of erythrocyte, Hb, platelet and leucocyte counts was calculated to age specific, minimum norm norm level. Black line at zero indicates the minimum-norm level, levels above are either within the range or, if too high, marked with a red circle.*

Hb - Hemoglobin

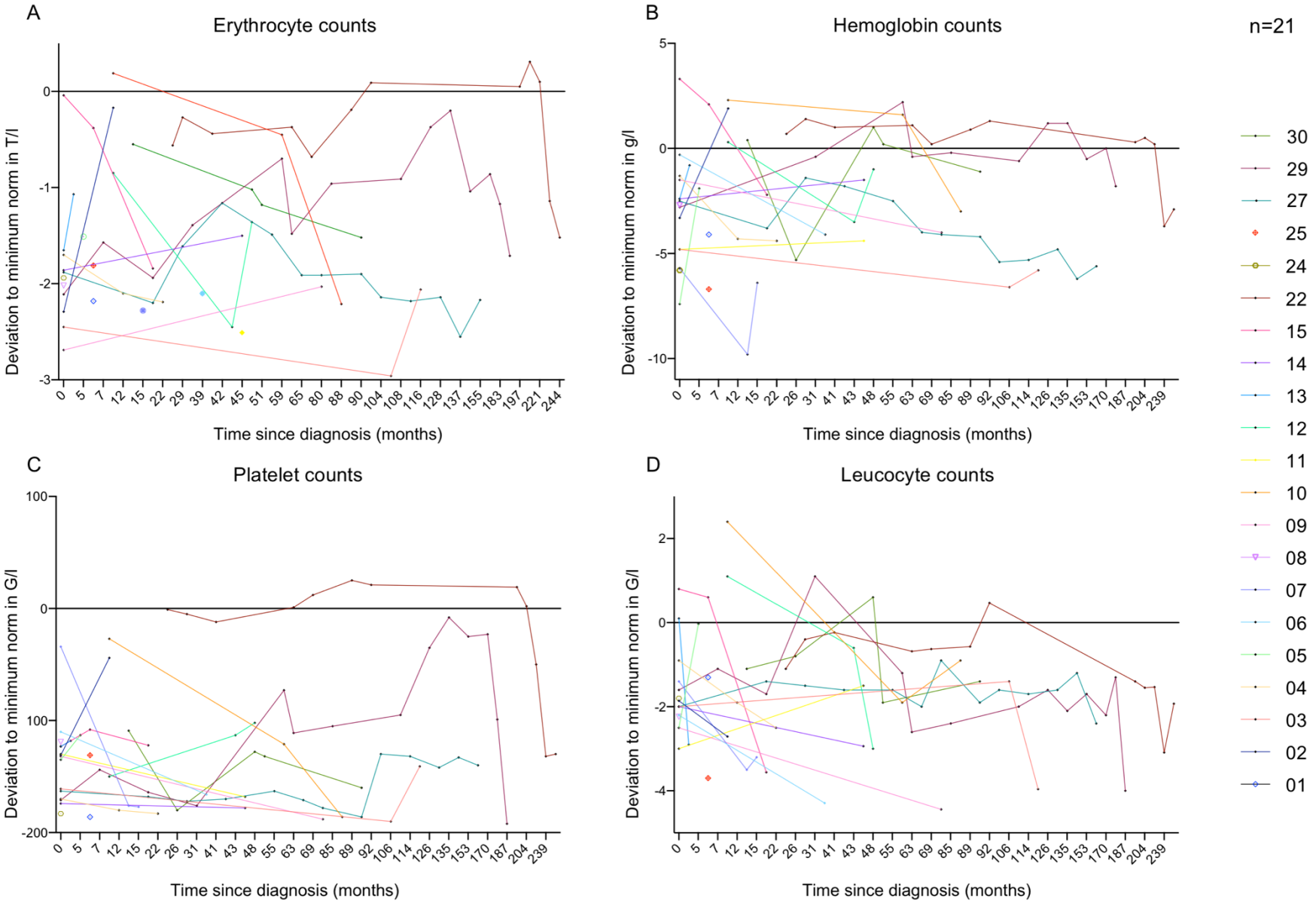


Figure 7b. Fanconi anemia: Blood levels over time since diagnosis until HSCT in transplanted patients. *The deviation of erythrocyte, hemoglobin, platelet and leucocyte counts was calculated according to the minimum level of age specific values. The black line at zero indicates the minimum-norm levels, levels above are within the norm range.*

HSCT – Hematopoietic stem cell transplantation

Hormone levels were evaluated in all FA patients. Thyroid stimulating hormone (TSH) levels could be obtained from 15 patients and are shown in *Figure 8*. The maximum normal TSH range was used as reference, the deviation was calculated regarding the age of each patient. Hypothyroidism was present in five patients, although individuals within the range may have undergone substitutional therapy with levothyroxine. Other endocrinological abnormalities rather than hypothyroidism were described in several patients, such as diabetes mellitus, growth hormone deficiency, hyperparathyroidism and osteopenia.

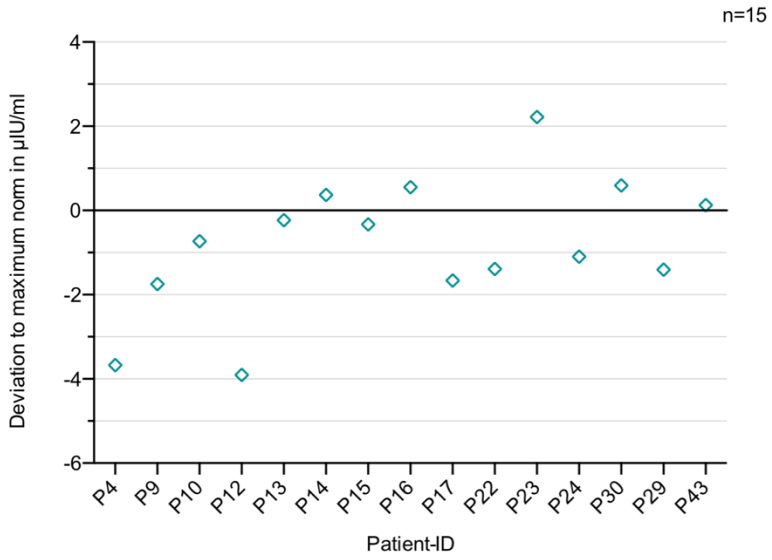


Figure 8. Fanconi anemia: TSH levels at diagnosis. *Line at zero indicates upper limit of age specific norm range. Levels underneath are within the normal range.*
TSH – Thyroid stimulating hormone

4.3.1 Hematopoietic stem cell transplantation and other therapeutic approaches in FA

Twenty-five patients required treatment due to progressive hematological abnormalities. Therapy regimes included blood transfusions (n = 19), androgen therapy (n = 9) and hematopoietic stem cell transplantation (HSCT, n = 21) (*Figure 9)*. Most patients received a combination of above mentioned therapies.

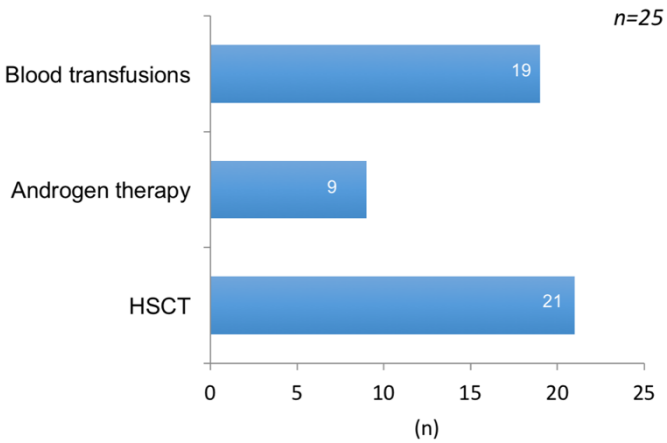


Figure 9. Fanconi anemia: Therapeutic approaches. *Patients received one, or combinations of two or all three therapies.*
HSCT – Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation was performed in 21 of 30 patients from 1990 to 2015 (see *Table 5* for detailed information about HSCT).

Median age at transplantation was 11.6 years (range, 4.2-29.2 years) with a median time since disease onset of 5.5 years (range, 0-14.1 years). Sixteen patients received blood transfusions prior to transplantation. In addition to this, patients 3, 9, 11, 27 and 30 were treated with androgens. Patient 29 received only androgen therapy prior to HSCT. In patients 3 and 11, androgen therapy was not sufficient and HSCT was carried out due to bone marrow failure (BMF), patients 9, 27 and 30 developed liver adenoma, thus therapy was stopped. In patient 29, androgen therapy was stopped due to signs of liver malfunction, while blood counts remained stable. Patients 27, 29 and 30 all developed myelodysplastic syndrome (MDS).

In total, 13 patients (61.9%) were transplanted due to BMF, five patients (23.8%) due to MDS, and three patients (4.3%) due to acute myeloid leukemia (AML).

Eleven patients (52.4%) received a matched transplant from a sibling (MSD), 10 patients (37.6%) received a transplant from a matched unrelated donor (MURD). Nineteen patients (90.5%) received bone marrow transplants (BM), two (9.5%) received peripheral stem cells (PSC), both patients transplanted with PSC died. Conditioning regimes were analyzed and divided in groups based on the combination of medication. Eight patients received a combination of fludarabine (Flu), busulfane (Bu), anti-thymocyte globulin (ATG) and Alemtuzumab (Campath®). Two patients with this regime received additional radiation therapy (one patient total body irradiation, the

other patient total lymphoid irradiation). Both irradiated patients died. One patient received a combination of Flu, Bu, ATG, and cyclophosphamide (CY). Three patients were treated with a combination Flu, Bu, and ATG and one patient with Bu, ATG and Cy. Another patient received ATG, Cy and additionally TLI. Five patients were treated with CY, two of whom received TLI.

The response to transplantation was monitored. Twelve patients (57.1%) had initial engraftment, two patients (9.5%) developed primary graft failure (rejection of graft) and five patients (23.8%) secondary graft failure (transient engraftment). No information could be obtained for another two patients (9.5%). As a consequence of poor engraftment rate, four patients (19%) were given a stem cell boost, one of these patients (4.8%) received an additional stem cell boost. Twelve of the transplanted patients (57.1%) developed graft versus host disease (GvHD) and the number of acute vs. chronic GvHD was distributed equally. Two (16,6%) of these patients developed acute and chronic GvHD. Information for one patient (4.8%) could not be obtained.

The overall survival of transplanted patients was 57.1% (n=12), nine patients (42.9%) died of the consequences of HSCT. Death causes included multi organ failure, sepsis and cGvHD. The initial diagnosis of five of the deceased patients was BMF, one patient had AML and three patients were transplanted because of MDS.

Table 5. Fanconi anemia: HSCT characteristics n=21

Patient-ID	Age at disease onset (years)	Age at HSCT (years)	HSCT (year)	Therapy prior to HSCT	HSCT indication	Donor type	Graft type	Conditioning regimen	Engraftment	Re-transplant/ stem cell boost	GvHD	Deceased/alive at last follow-up (year)	Cause of death
1	1.3	8.1	2009	BT	BMF	MURD	BM	Flu, Bu, Sero + TBI	–[1]	+	–	†	CMV pneumonia
2[4]	1.2	6.3	2007	BT	BMF	MURD	PSC	Flu, Bu, Sero + TLI	–[2]	+	–	†	MOF
5	3	5.5	2007	BT	AML	MSD	BM	Flu, Bu, Sero[6]	+	–	a/c	2015	–
25	16	16.7	2005	BT	AML	MSD	BM	Flu, Bu, Sero[6]	+	–	a/c	†	GvHD of gut, ileus
3	9.6	17.9	2005	BT, AG	BMF	MURD	BM	Flu, Bu, Sero[6]	–[2]	+[3]	a	†	Pneumonia, MOF
8	0.3	11.1	2003	BT	MDS	MSD	BM	Flu, Bu, Sero[6]	+	–	c	2014	–
9	6.2	12.5	2003	BT, AG	BMF	MURD	BM	Flu, Bu, Sero[6]	+	–	–	2015	–
15	1.5	4.2	2004	BT	BMF	MSD	BM	Flu, Bu, Sero[6]	+	–	–	2014	–
7	1.4	14.7	2005	BT	AML	MSD	BM	Flu, Bu, Sero[7]	+	–	c	2011	–
10	7.4	9.3	2004	BT	BMF	MURD	BM	Flu, Bu, Sero[7]	–[2]	–	a/c	2015	–
27	15	23.4	2014	BT, AG	BMF	MURD	BM	Flu, Bu, Sero[7], CY	n.a.	–	a	†	n.a.
29	16	21	2006	AG	MDS	MURD	PSC	Flu, Bu, Sero[7]	–[2]	–	a	†	MOF, septic shock
22	n.a.	29.2	2015	–	MDS	MURD	n.a.	n.a.	n.a.	–	a	†	Liver failure
30	12.6	15.7	2009	BT, AG	MDS	MURD	n.a.	n.a.	–[1]	+	n.a.	†	Infection
6	4.3	16.3	1998	–	MDS	MSD	BM	Bu, Sero[7], CY	+	–	–	2013	–
14	4	11.6	1995	BT	BMF	MSD	BM	ATG, CY + TLI	+	–	–	2013	–
4	3	9.6	1994	BT	BMF	MSD	BM	CY + TLI	–[2]	–	a/c	†	GvHD of lung
11	3.6	8.4	1991	BT, AG	BMF	MURD	BM	CY	+	–	c	2006	–
12[5]	6.9	6.9	1990	BT	BMF	MSD	BM	CY	+	–	c	2016	–
13	1.3	5.5	1993	–	BMF	MSD	BM	CY + TLI	+	–	–	2013	–
24	0.2	14.3	1998	–	BMF	MSD	BM	CY	+	–	–	2015	–
n = 21	Median 3.8; range (0.2 - 16)	Median 11.6; range (4.2 - 29.2)			BMF n = 13 MDS n = 5 AML n = 3	MSD n = 11 MURD n = 10						† n = 9 alive n = 12	

Detailed information to HSCT for each patient individually. + yes; – no;

[1] primary graft failure, [2] secondary graft failure, [3] two stem cell boosts, [4] CD19/CD3 depletion, [5] developed SCC, [6] Serotherapy with ATG/Alemtuzumab (Campath®), [7] Serotherapy with ATG

a – acute GvHD, a/c– acute and chronic GvHD, AG – androgen therapy, AML – acute myeloid leukemia, ATG – anti-thymocyte globulin, BM – bone marrow, BMF – bone marrow failure, BT – blood transfusion, Bu – Busulfan, c – chronic GvHD, Cyclo – Cyclophosphamide, (a/c)GvHD – (acute/chronic) graft vs. host disease, HLA – human leucocyte antigen, HSCT – hematopoietic stem cell transplantation, MDS – myelodysplastic syndrome, MSD – matched sibling donor, MURD – matched unrelated donor, PSC – peripheral stem cells, SCC – squamous cell carcinoma, Sero – Serotherapy, TBI – total body irradiation, TLI – total lymphoid irradiation, † – deceased

To address if the likelihood of patients becoming candidates for HSCT increases with age, a correlation analysis between age of diagnosis and timespan until HSCT was performed. Statistical analysis was carried out and showed a trend, that the older the patients were diagnosed, the smaller the timespan until HSCT (r^2-value of 0.13). This was found regardless of the type of underlying hematological disorder. (*Figure 10*).

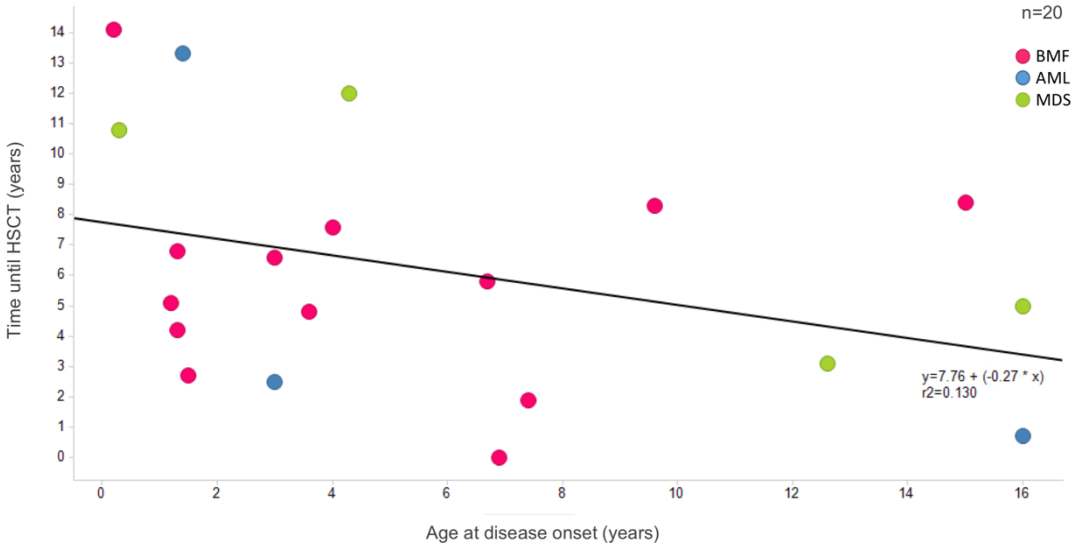


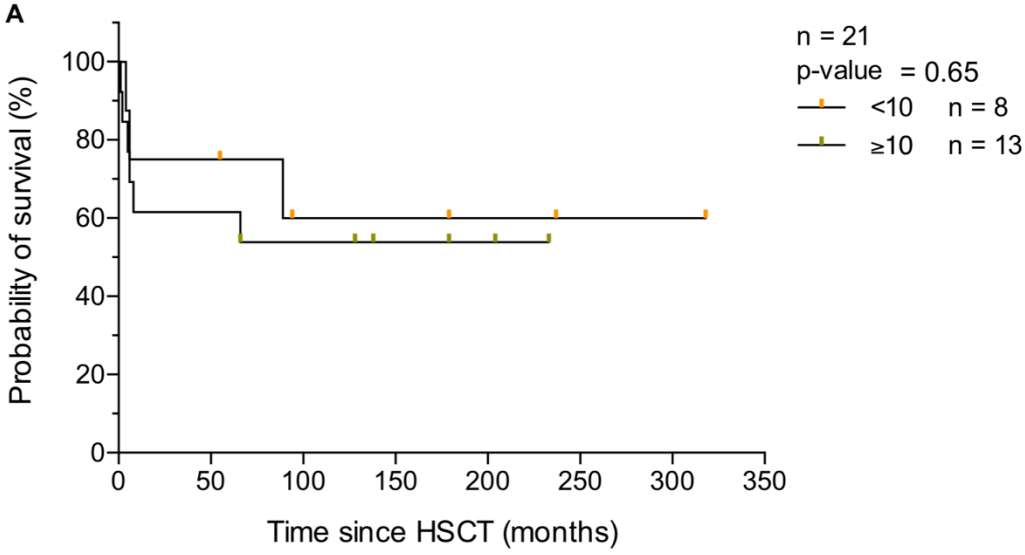
Figure 10. Fanconi anemia: Time from age at disease onset until HSCT. *Patients were divided in three groups by indication of transplantation. Correlation coefficient (r2) and curve characteristics (y) are noted in black underneath the line.*
AML – acute myeloid leukemia, BMF – bone marrow failure, HSCT – Hematopoietic stem cell transplantation, MDS – myelodysplastic syndrome.

Previous reports suggest a higher probability of survival after HSCT with an intervention in patients younger than 10 years old, whereas no significant differences regarding the development of complications have been described.[141] A worse survival at older age has also been described in HSCT under different circumstances (SCID, severe aplastic anemia).[142,143] Thus, survival after transplantation was analyzed to evaluate if age at HSCT correlated with survival status (*Figure 11*). After HSCT, 57.1% of patients (n=12) survived with a median follow-up period of 158.5 months (range, 55-318 months). Meanwhile, 42.9% of patients (n=9) died of the consequences of HSCT, with a median survival of six months (range, 1-89 months) after transplantation. The average age at HSCT of the surviving patients was 10 years, while the average age of deceased patients was 16.4 years. The correlation analysis was done dividing the transplanted patients in two groups: older and younger than 10 years of age. Although no statistical significance was found (p-value 0.65), the results show a trend and are concordant with previous findings.

To evaluate if the risk of developing complications after HSCT increases with age ≥10 years at time of transplantation, a correlation between the two groups (younger vs. older than 10 years) and development of GvHD was done. The results showed no statistical significance (p-value 1). The average age of patients who presented complications was 13.4 years, whereas the group of patients free of complications had an average age of 10.7 years (see *Figure 12*).

Other studies suggest, that donor relation plays a crucial role in HSCT outcome.[144] The survival of the group of patients transplanted using MSD was 80% after 180 months, whereas the patients transplanted using MURD had an overall survival of 30% at this time (p-value = 0.009). These results are in agreement with findings of previous studies.[144] The development of complications (graft failure and GvHD) in patients using MSD and MURD was analyzed and are shown in *Figure 12*. 54% of patients transplanted with MSD-bone marrow (n=6) developed complications, whereas in 90% of patients transplanted with MURD-bone marrow, complications were found (p-value = 0.14). The number of patients developing GvHD was the same in both groups (n = 6 respectively). Six out of 8 evaluable patients transplanted with MURD-bone marrow developed graft failure, however this was found in only one transplanted patient with MSD-bone marrow (p-value = 0.006).

A

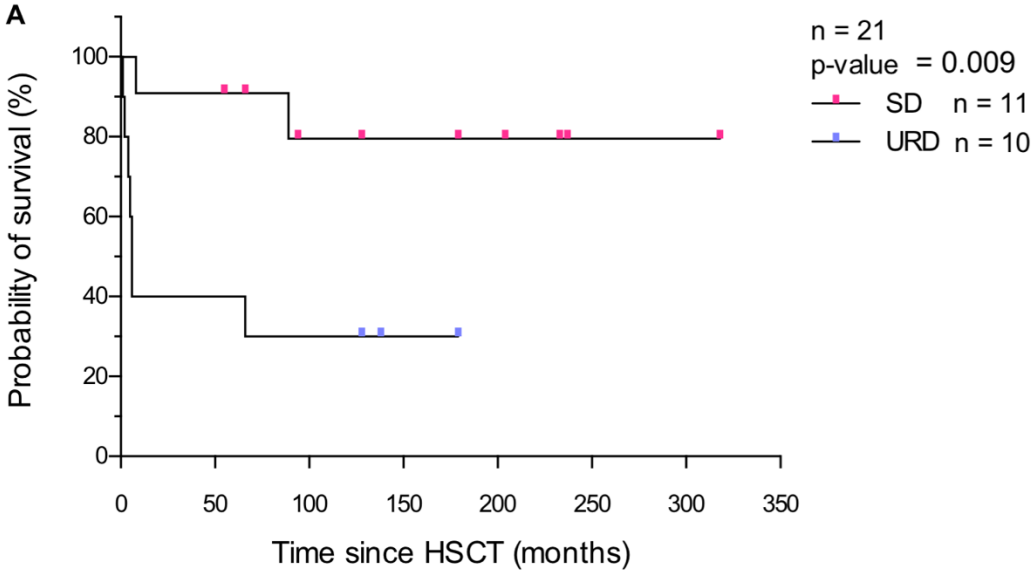


B	Age at HSCT (n)		Total (n)	p-value
	<10 years	≥10 years		
Complications	6	9	15	1
No complications	2	4	6	

Figure 11. Fanconi anemia: (A) Survival after HSCT in correlation to age at HSCT. *Patients were divided in 2 groups according to age at HSCT: ≥10 years and <10 years old. Analysis was performed using the Mantel-Cox test.* ***(B) Development of complications in correlation to age at HSCT.*** *Age groups of ≥10 and < 10 years old were used to calculate correlation using Fisher's test.*

FA – Fanconi anemia, HSCT – hematopoietic stem cell transplantation

A



B	SD (n)	URD (n)	Total (n)	p-value
GvHD *	6	6	12	0.66
No GvHD	5	3	8	
Graft failure *	10	2	12	0.006
No graft failure	1	6	7	

Figure 12. Fanconi anemia: (A) Probability of survival after HSCT in SD vs. URD. Correlation analysis was performed using Mantel-Cox test. **(B) Correlation of SD vs. URD and survival, complications, development of GvHD and graft failure.** Correlation analysis of transplanted patients using SD vs. UDR and development of complications and specific complications. Fisher's exact test was used to analyze data.

* information could not be obtained for all patients

GvHD – graft vs. host disease, HSCT – hematopoietic stem cell transplantation, SD – sibling donor, URD – unrelated donor.

FA patients have been described to develop malignancies with a 500 to 700-fold higher risk than the general population.[38,62,63] In this thesis, 17 malignancies were found in 12 FA patients (36%) (*Figure 5)*, details are shown in *Figure 13.* The most frequent malignancy was myelodysplastic syndrome (n=7). Patient 8 developed an MDS relapse one year after HSCT and received re-transplantation. Median age at manifestation in these patients was 16 years (range, 10.8-29 years). AML was found in three patients, two of whom had previously been diagnosed of MDS. Median age at AML onset was 14.7 years (range, 4.7-16.6 years). Cytogenetics and additional information for patients with MDS and AML are shown in *Table 6*.

Three patients developed liver adenoma during androgen therapy. Detailed information regarding durance and dosage of administration was available only for a minority of patient. Median age at onset was 12.3 years (range, 11.6-19.1 years).

Patient 26 was diagnosed of hepatoblastoma at age 4.7 and died with 4.8 years of its consequences. Patient 12, a previously transplanted patient, developed SCC at age 33.3, 26.4 years after HSCT, and received surgery.

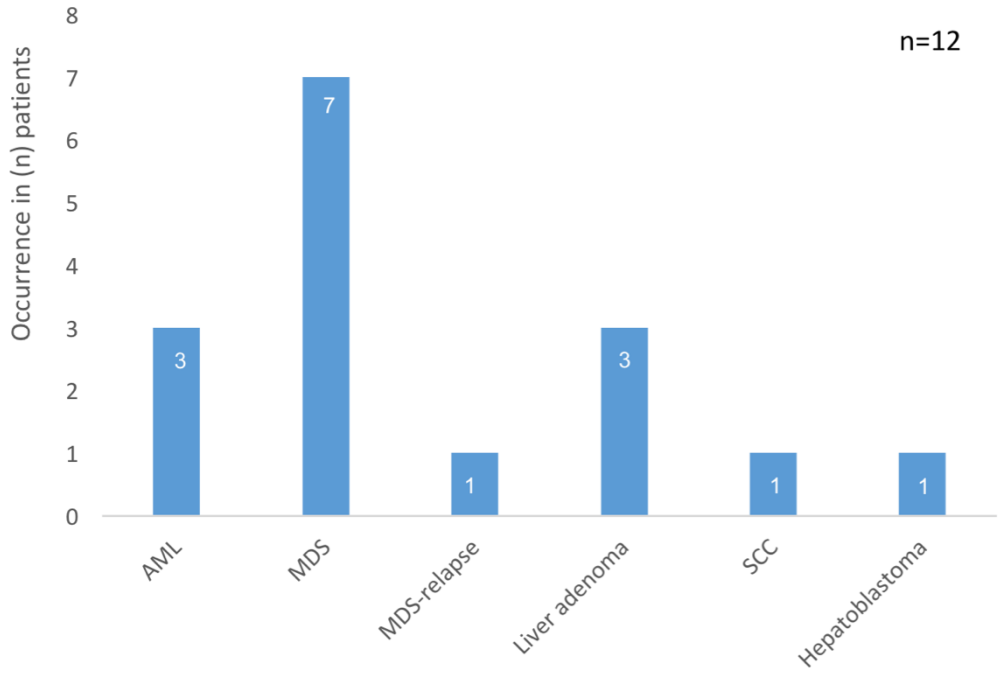


Figure 13. Fanconi anemia: Occurrence of neoplasms*. Multiple neoplasms in one patient are possible.*
AML – acute myeloid leukemia, MDS – myelodysplastic syndrome, SCC – squamous cell carcinoma

Table 6. Fanconi anemia: Cytogenetics and additional information for patients with hematological malignancies.

Patient-ID	Malignancy	Age	Cytogenetics/additional information	n=10
6	MDS	16	Monosomy 7	
7	MDS	14.4	RAEB1, 6% blasts	
8	MDS	10.8	RAEB1, 8% blasts, no clonal aberrations	
30	MDS	15.5	t(1:4) del 5q del 13q	
22	MDS	29	RAEB2	
25	MDS	16.6	Monosomy 7, structural changes in chromosomes 12, 13	
29	MDS	20.6	46, XX, add(3)(q29),del(21)t(1.21)(q10.p10); pseudodiploid clone with structural chromosomal abnormalities, characteristic for MDS in FA	
5	AML	4.7	Bone marrow blasts 35%, peripheral blasts 2%	
7	AML	14.7	Bone marrow blasts 20%	
25	AML	16.6	Blasts 40%	

AML – Acute myeloic leukemia, MDS – myelodysplastic syndrome

Figure 14 shows a summary of all patients over the course of time, divided in three categories: (A) age at onset of symptoms, diagnosis and death, (B) age at occurrence of neoplasm, showing all neoplasms including primary diagnosis of MDS, having then transformed into AML within a short time, (C) age at start of therapy. Age at last follow-up and presumed age (September 2016) were included in category (A).

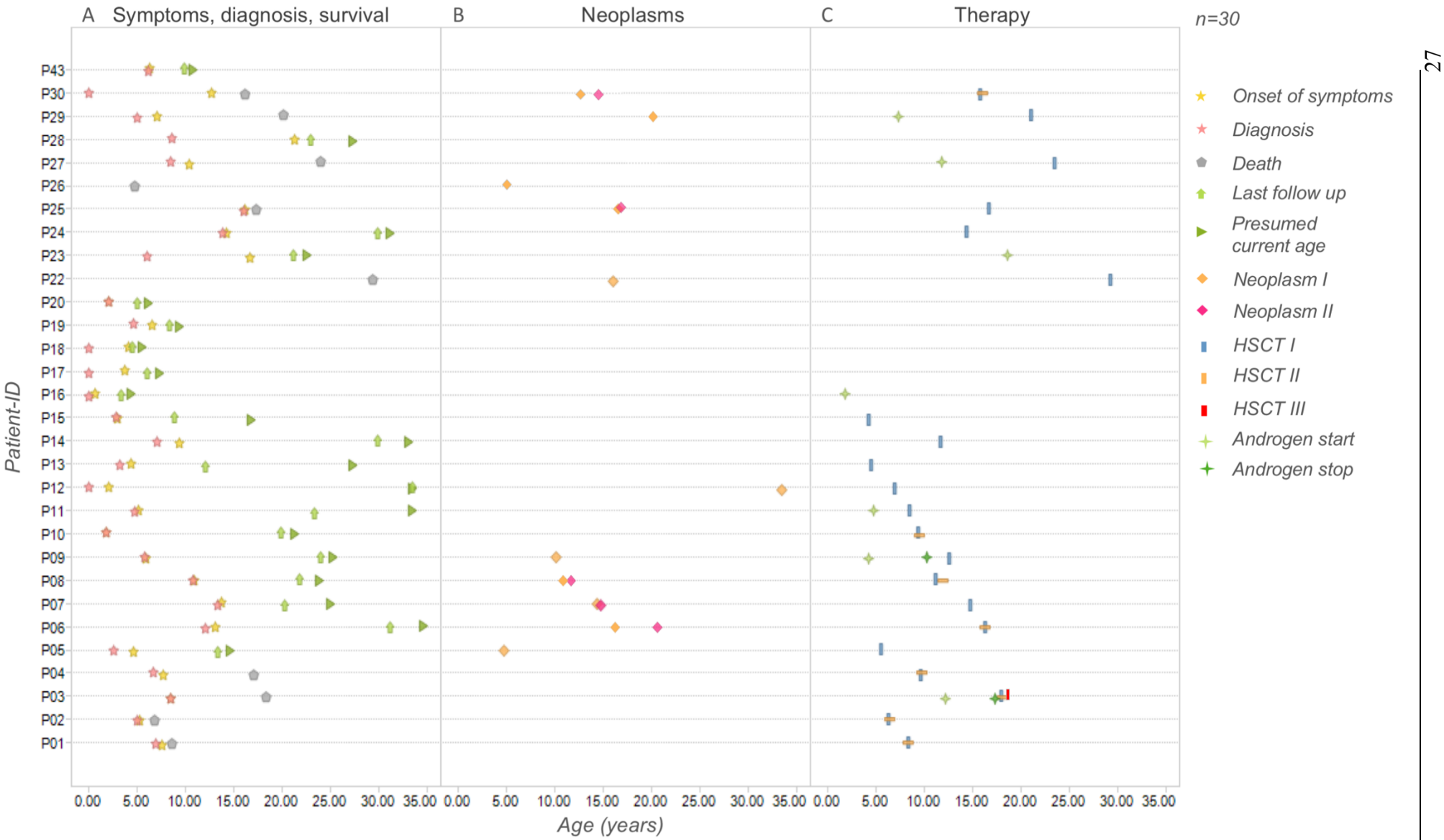


Figure 14. Fanconi anemia: Patients over the course of time. *(A) Symbols indicating age at symptom onset, diagnosis, death, last follow up and presumed age (Sept.2016) (B) Age at occurrence of neoplasms. Occurrence of multiple neoplasms in one patient are possible. (C) Age at start/stop of androgen or stem cell therapy (up to three transplants in one patient).*

AML – acute myeloid leukemia, HSCT – hematopoietic stem cell transplantation, MDS – myelodysplastic syndrome

4.4 Ataxia telangiectasia (ATM)

The group of ATM patients consisted of 10 individuals, sex ratio was eight females to two males. Median age at diagnosis was 7.7 years (range, 1.4 – 18.8 years) with a diagnostic delay of 5.6 years (range, 0.4-17.5 years). Physical abnormalities could be evaluated in seven patients and were present in five (*Figure 15*). One patient had microcephaly, telangiectasis was described in four patients. Three patients showed abnormalities of the skin, hypopigmentation, café au lait spots and hair pigment naevus. One patient had hydronephrosis and failure to thrive. One other patient showed telangiectasis of the back (see *Figure 16)*. Detailed information for each patient can be found in the appendix, *Table 10.*

Alpha-fetoprotein (AFP) levels were available for five patients and were elevated in all five of them (*Figure 17).* Immunoglobulin counts were available in 7 patients for at least one occasion. Most patients presented with IgA-deficiency. Ig-therapy was administered in patients 36 (start 20 months after diagnosis), 37 (start 32 months after diagnosis), 38 (start 58 months after diagnosis), 41 (start 11 months after diagnosis), 45 (start 217 months after diagnosis) and 46 (start 18 months before diagnosis) with substances such as IG Vena®, Intratect®, Endobulin® and Vivaglobin® (Hizentra®).

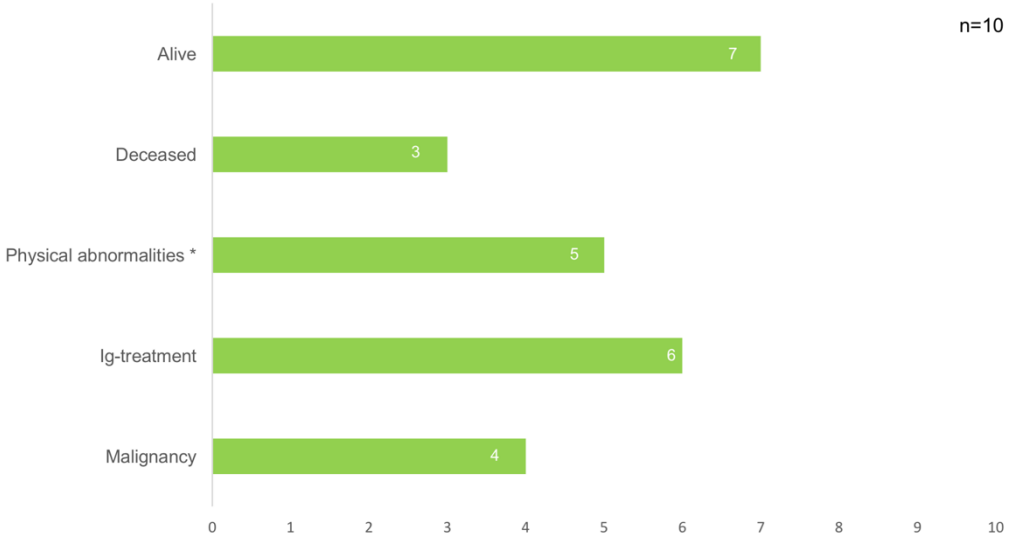


Figure 15. Ataxia telangiectasia: Patient characteristics.
** Information of three patients about physical abnormalities could not be obtained.*

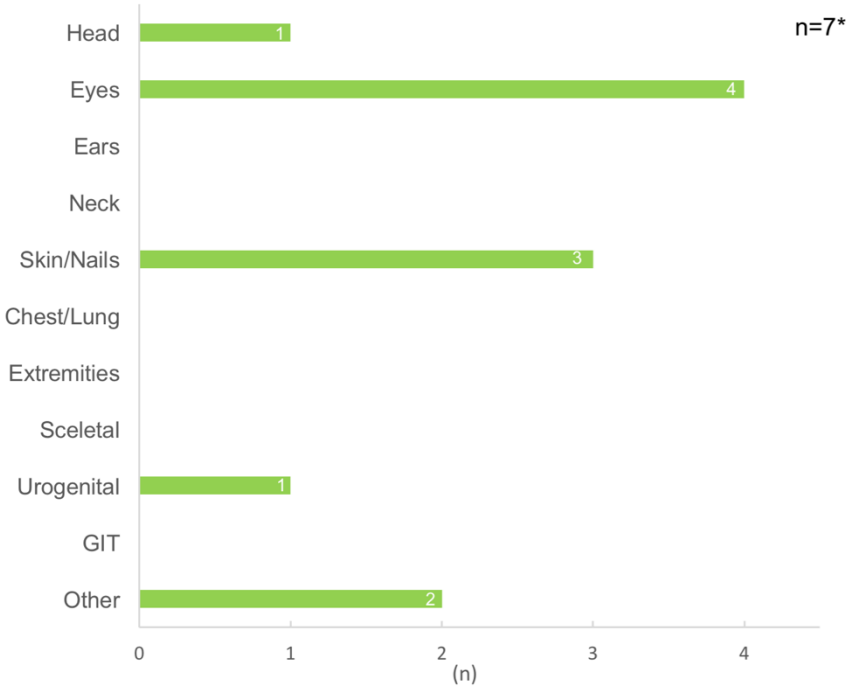


Figure 16. Ataxia telangiectasia: Physical abnormalities. *Occurrence of physical abnormalities at different body sites.*
** information was available for only 7 of 10 patients*
ATM – Ataxia telangiectasia, GIT – gastrointestinal tract

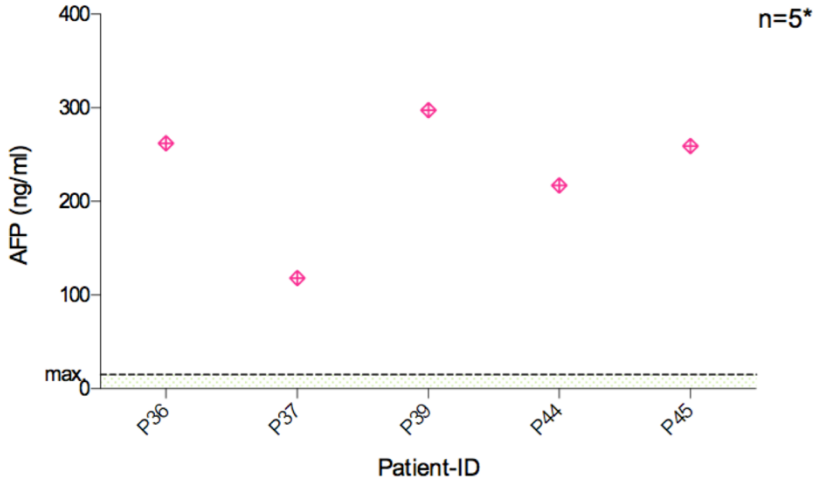


Figure 17. Ataxia telangiectasia: AFP levels. *Black line indicates normal maximum range.*
**AFP levels could not be obtained for five more patients.*
AFP – Alpha-fetoprotein, ATM – Ataxia telangiectasia

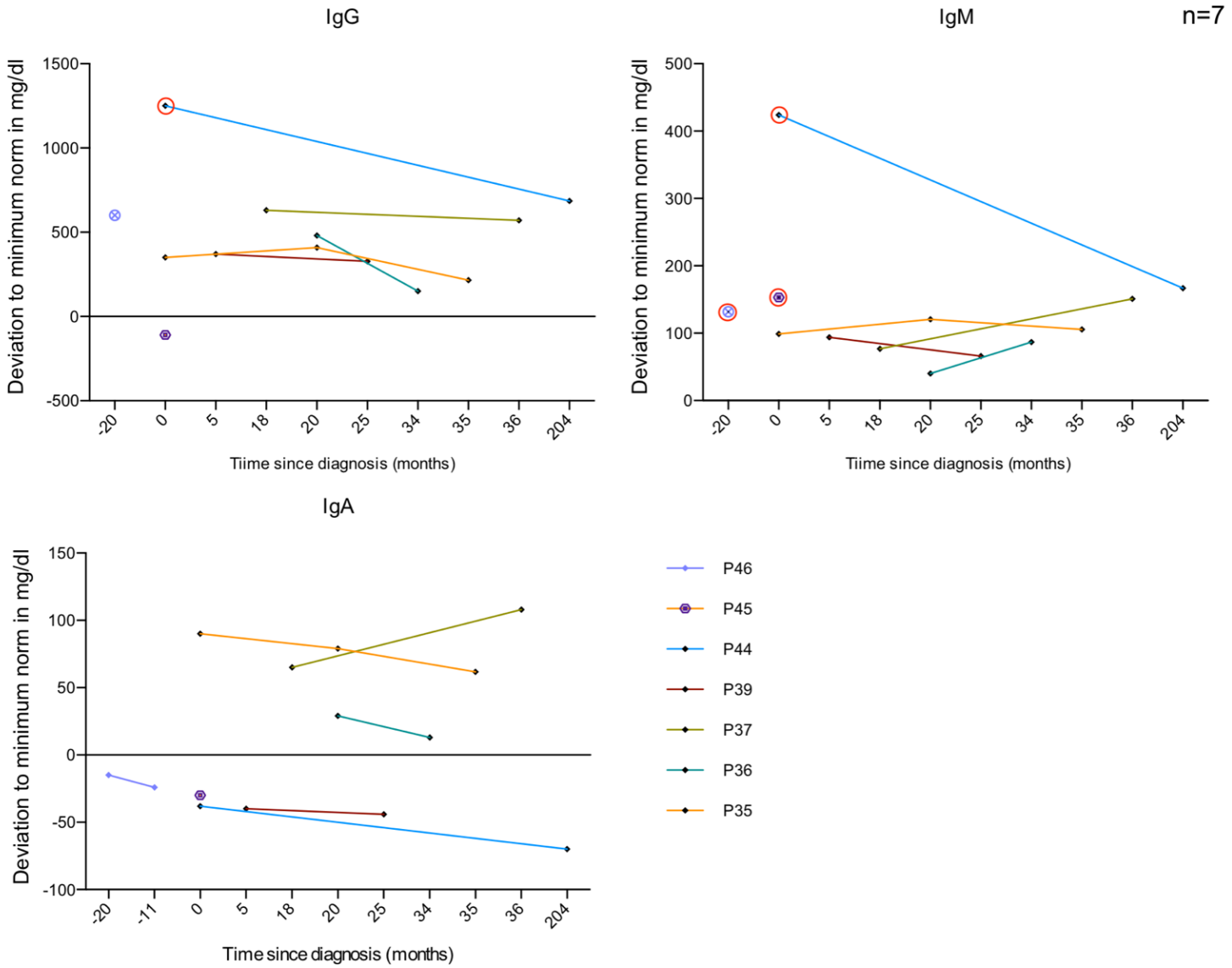


Figure 18. Ataxia telangiectasia: Immunoglobulin levels. *All values reflect the deviation of the actual count towards the age specific minimum normal level (line at zero). Values below are too low, values above are within the range or, if circled red, above the normal range. Values are shown in months before (minus) or months after diagnosis.*

ATM – Ataxia telangiectasia, Ig – Immunoglobulin

A full clinical description was compiled and can be found in *Table 7*. Detailed information about genetic features, type of immunodeficiency and development of neurological symptomatic were collected. Information about genetic mutations was available for six patients. Patient 36 showed compound heterozygous mutations (c.3850delA, exon 28; c.6095G>A, exon 43), the same mutations were found in patient 37, sibling to 36. Patient 39 had a homozygous base exchange (c.4236+4>G). A homozygous mutation in 381delA was found in patient 44, whereas patient 45 showed a mutation in R2443X, exon 52. Patient 46 had a hemizygous base exchange in c5309C<A and a heterozygous deletion of exon 34-46 (x-5006-?_6807+?del).

The median age at onset of symptoms was one year (range, 0.8-3.2 years). In patients 35, 36, 37, 39, 40, 41, 42 and 46 ataxia became apparent, in patients 38 and 45 recurring infections were the leading symptom. B- and T-cell deficiency was present in patients 36, 37, 39 and 44, patient 45 showed T-cell deficiency. For patients 35, 40, 41 and 46 no information was available.

Four patients developed malignancy, B-non-Hodgkin lymphoma occurred in patient 36 (at 20.3 years) and 45 (at 13.3 years), T-PLL in patient 44 (at 24.2 years), patient 46 was diagnosed of medulloblastoma at age 3.8. Patient 44 died of liver failure (age 25.2), patients 45 (age 20.9) and 46 (age 5.7) of multiple organ failure.

Table 7. Ataxia telangiectasia: Characteristics of patients and their immunodeficiency. n=10

Patient-ID	Mutation	Age at diagnosis (years)	Age at first symptoms (years)	First symptoms	Age at start Ig-therapy (years)	Age at last follow up/ death (years)	Diagnosis	Humoral immunity	Cellular immunity	Infections during therapy	Obersvations	Malignancy	Cause of death
35	n.a.	9.6	n.a.	Ataxia	No therapy	16.7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Alive
36	Compound heterozygous; c.3850delA, exon28; c.6095G>A, exon43	18.8	1.3	Ataxia, dystonia	20.4	22.8	Suspicion of AT after long years of known neurological problematic	Hypogamma-globulinaemia	B- & T-cell deficiency	No severe infections	Hyper-phosphatemia; first neurological symptoms at age 1.3: ataxia, dystonia, dyslalia, hypersalivation, relatively steady over time	B-non Hodgkin lymphoma (age 20.3)	Alive
37	Compound heterozygous; c.3850delA, exon28; c.6095G>A, exon43	14.5	1	Ataxia, dystonia, recurring respiratory tract infections	17.4	18.3	Older sibling with same syndrome	Dysgamma-globulinaemia	B- & T-cell deficiency	No severe infections	Worsening of ataxia, walking without aid at 16.8 years not possible	–	Alive
38	n.a.	1.1	n.a.	Recurring infections	5.3	5.4	n.a.	n.a.	n.a.	n.a.	n.a.	–	Alive
39	Homozygous c.4236+4A>G	8.3	1	Ataxia	No therapy	11.4	n.a.	Dysgamma-globulinaemia (IgA deficiency, Hyper-IgM)	B- & T-cell deficiency	–	Wheelchair at age 9	–	Alive
40	n.a.	1.8	1	Ataxia	No therapy	3.4	n.a.			–		–	Alive
41	n.a.	7.4	1.8	Recurring infections, ataxia	8.3	8.8	n.a.	n.a.	n.a.	n.a.	n.a.	–	Alive
44	Homozygous c.381delA	7.8	1.8	Ataxia, motordevelopmental delay	No therapy	† 25.2	n.a.	Hypogamma-globulinaemia (IgG2, IgG4, IgA deficiency)	B- & T-cell deficiency	–	n.a.	T-PLL (age 24.2)	Liver failure
45	R2443X exon 52	7.7	3.2	Recurrent infections (ear)	8.9	† 20,9	n.a.	Hypogamma-globulinaemia (IgG1, IgG2, IgG4 deficiency)	T-cell deficiency	–	Ataxia worsening over course of time	B-non Hodgkin lymphoma (age 13.3)	MOF
46	Hemizygous base exchange c5309C>A; heterozygous deletion of exon 34-46 (x.5006-?_6807+?del)	5.4	0.8	Ataxia	3.9	† 5,7	Severe radio/chemo-sensitivity in treatment for medullo-blastoma	Hypogamma-globulinaemia (IgA deficiency)	n.a.	n.a.	n.a.	Medulloblastoma (age 3.8)	MOF

Ig – Immunoglobulin, MOF – multiple organ failure

4.5 Nijmegen breakage syndrome (NBS)

The group of NBS patients consisted of six individuals, treated in Graz and Vienna (see *Figures 3* and *4*).

All patients survived, with a sex ratio of two females and four males. The median age at diagnosis was 8.3 years (range, 0-25 years) with a diagnostic delay of 8.1 years (range, 0-18 years). Median age at immunological symptom onset was 3.5 years (range, 0-7 years). Physical abnormalities were described in all patients (*Figure 19)*. Microcephaly was the most frequent abnormality and present in six patients, two patients showed abnormalities of the eyes, such as microphthalmia, hypotelorism and protrusio bulbi. One patient showed an abnormality of the ears, hypakusis. In two patients, abnormalities of the skin such as café-au-lait spots, hypopigmentation and palmo-plantar hyperkeratosis were present. As an abnormality of the urogenital tract, hydronephrosis was found in one patient. Anal and rectal atresia were found as gastrointestinal abnormalities in one patient as well. (see *Figure 20*). Detailed information for each patient can be found in the appendix, *Table 7*.

Immunoglobulin levels are presented in *Figure 21*. Values were obtained before diagnosis throughout medical follow-up and are depicted as *months since diagnosis* (negative numbers for counts before diagnosis). The reference levels for immunoglobulin counts vary with age, therefore, the deviation towards the minimum norm range for IgG, IgG-subclasses, IgA and IgM was calculated. Previous studies have described a connection between the occurrence of malignancies and elevated IgM-levels in NBS patients.[118,124] Thus, levels outside the maximum norm were marked with a red circle. IgA deficiency was found to be the most common among the group of patients included. Elevated IgM levels were detected in three patients.

Patients 21, 31, 32, 33, and 34 received Immunoglobulin supplementation therapy (Ig-therapy) due to symptomatic immunodeficiency. Substances such as Gammanorm®, Vivaglobin® and IG-Vena® were used for Ig-therapy. Patient 21 showed signs of immunodeficiency and was started on Ig-therapy 11 months prior to diagnosis of NBS. In patient 31 Ig-therapy was started 95 months after diagnosis, 11 months after diagnosis in patient 32, 10 months after diagnosis in patient 33 and 28 months after diagnosis in patient 34. Median age at start of Ig-treatment was 8.3 years (range, 0.8-10.6 years). Only patient 33 showed persistent respiratory infections with high fevers

during Ig-substitution (*see Table 7).* Patient 47 did not require Ig-Therapy. None of the patients received HSCT.

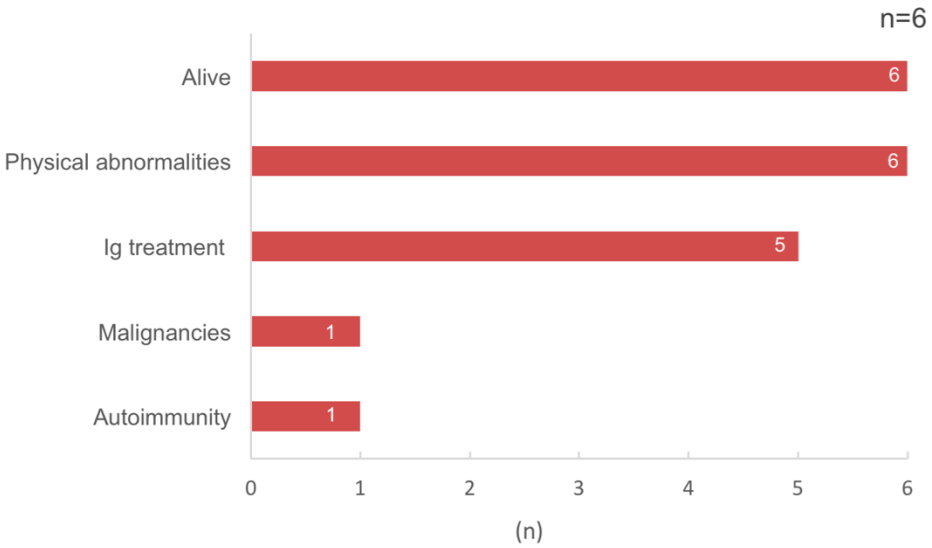


Figure 19. Nijmegen breakage syndrome: Patient characteristics. *Survival status, occurrence of physical abnormalities, malignancies and autoimmunity, Ig-treatment*

Ig – Immunoglobulin, NBS – Nijmegen breakage syndrome

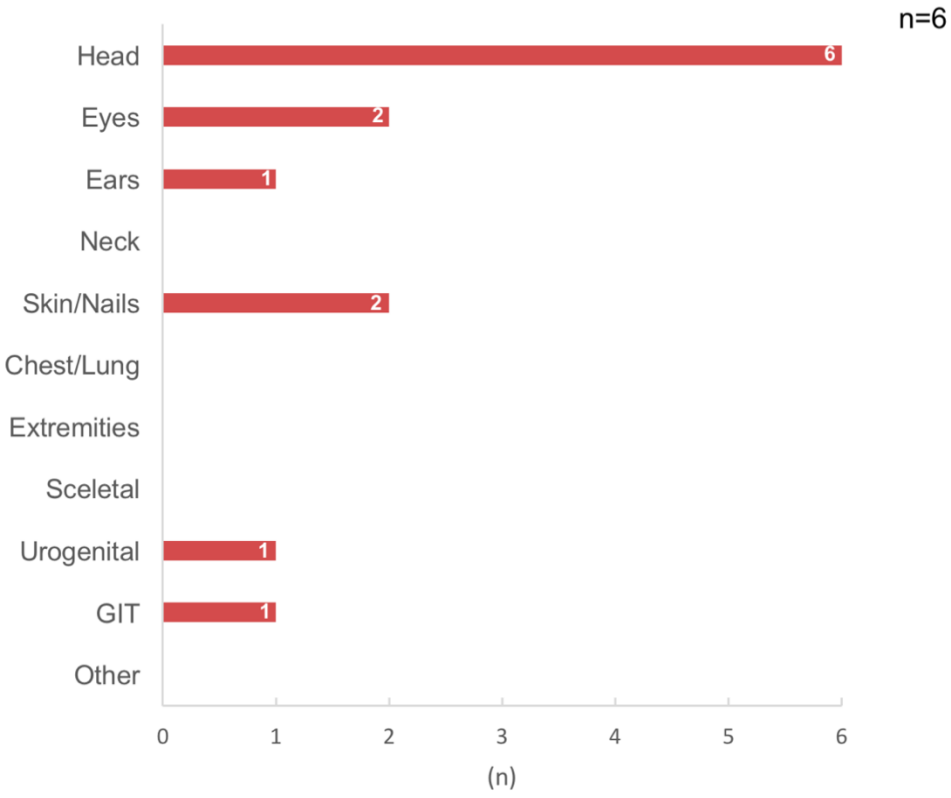


Figure 20. Nijmegen breakage syndrome: Physical abnormalities. *Occurrence of physical abnormalities of all patients at different body sites.*
GIT – gastrointestinal tract, NBS – Nijmegen breakage syndrome

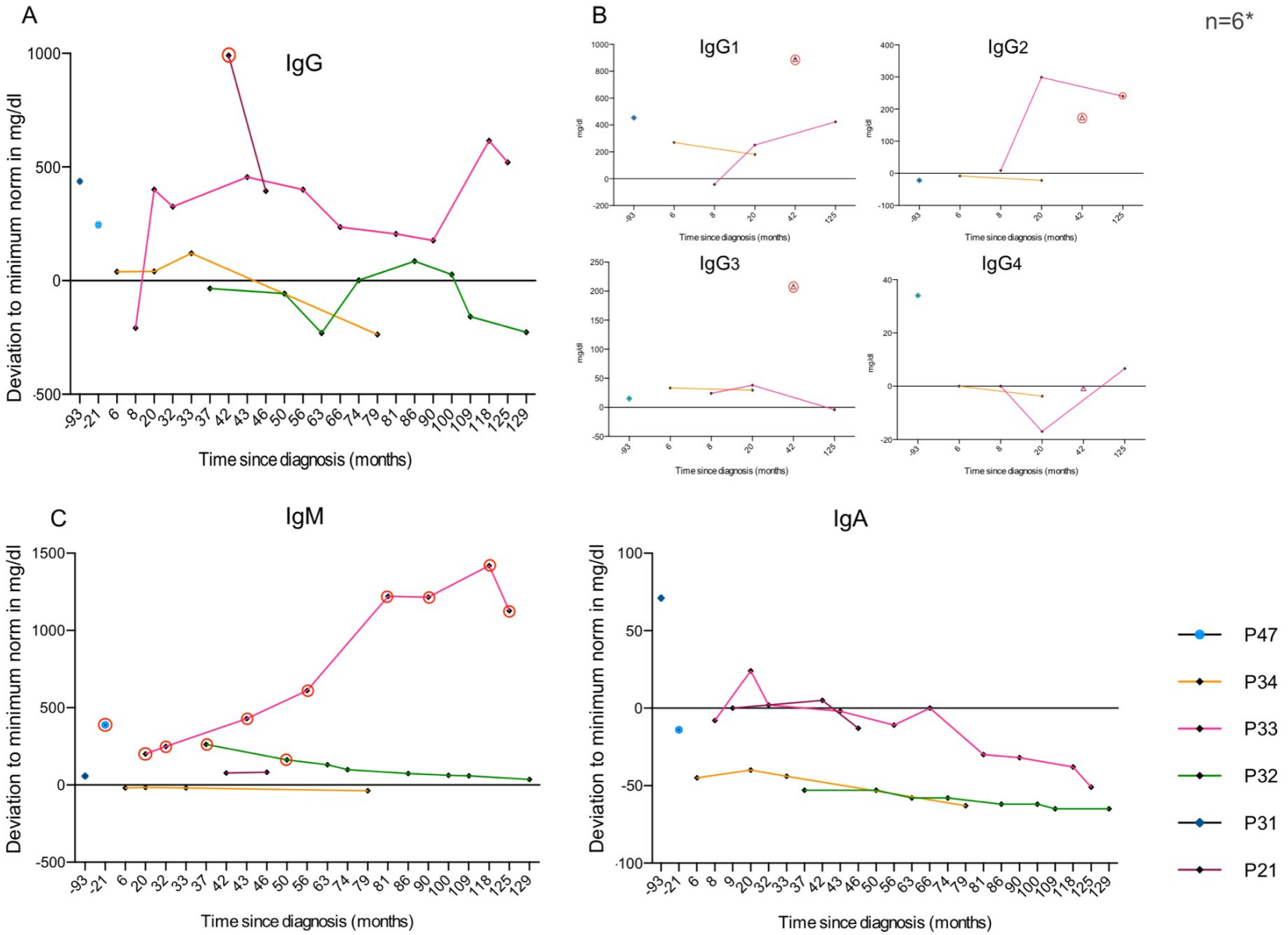


Figure 21. Nijmegen breakage syndrome: Immunoglobulin levels*. All values reflect the deviation of the actual value towards the age specific minimum normal level (line at zero). Values below are too low, values above are within the range or, if circled red, above the normal range.*

** (B) IgG subclasses n=4*

Ig – Immunoglobulin, NBS – Nijmegen breakage syndrome

The full clinical description of NBS patients is summarized in *Table 8*. Information regarding genetics, symptoms leading to diagnosis, therapy, humoral and cellular immunity, observations and malignancy were collected. All patients showed a mutation in the NBN gene, detailed information regarding the type of mutation was only found in four patients. Three patients showed the typical deletion of 657del5, whereas one patient showed a heterozygous missense mutation of R215W. Compound heterozygosity was suspected in this patient, but no second mutation could be identified.

Patients 21, 32 and 33 were diagnosed through a medical work-up for severe microcephaly. In patients 31 and 34 recurring infections lead to the suspicion of an immunodeficiency, thus medical examination was carried out. Patient 47 was diagnosed after showing severe chemo-sensitivity during treatment for B-non-Hodgkin lymphoma (B-NHL).

Humoral immunity was impaired in all patients, cellular immunity in five individuals. T-cell deficiency was present in all of them, B-cell deficiency was described in only two patients. In patient 47 no reports of cellular immunodeficiency were found. Developmental delay was described in two patients. Patient 47 showed a history of chronic juvenile polyarthritis and received immunosuppressive therapy over more than 15 years. The same patient was the only one of the group of NBS patients to develop a malignancy. B-NHL was diagnosed at age 25. Since the diagnosis of NBS was not yet known at that time, the patient received standard chemotherapy treatment following CHOP protocols. After having diagnosed the patient with NBS, chemotherapy was modified.

Table 8. Nijmegen breakage syndrome: Characteristics of patients and their immunodeficiency

n=6

Patient-ID	Gene / Locus	Mutation	Age at diagnosis (years)	Age at first immunological symptoms (years)	Age at start IG-Therapy (years)	Age at last follow up (years)	Symptoms leading to diagnosis	Humoral immunity	Cellular immunity	Infections during therapy	Obersvations	Malignancy
31	NBN	heterozygous BE in R215W	16.3	5	8.3	22.5	Recurring infections of upper and lower respiratory tract --> supicion of immunodeficiency	Hypogammaglobulinaemia	T-cell deficiency	No severe acute infections, chronic bronchitis	Hypothyreosis	–
32	NBN	homozygous 657del5	8.9	n.a.	9.8	19.7	Microcephaly work-up	Agammaglobulinaemia	B- & T-cell deficiency	No severe infections	Normal psychomotor development	–
33	NBN	homozygous 657del5	0	0	0.8	10.8	Suspicious pregnancy - prenatal growth retardation; at birth microcephaly, bird-like face --> work-up	IgA deficiency, Hyper IgM syndrome	T-cell deficiency, oligoclonal TCR repertoire	Respiratory tract infections + fever	Recurrent respiratory infections, hypoplastic brain parenchyma;	–
34	NBN	homozygous 657del5	8.3	1	10.6	16.4	Recurring respiratory tract infections --> work up	Hypogammaglobulinaemia	T-cell deficiency	Almost no infections	Developmental delay, limited cognitive abilities	–
21	NBN	homozygous 657del5	4.4	3.5	3.5	4.5	Microcephaly work-up	IgG1, IgG2, IgA deficiency	B- & T-cell deficiency	No infections since start of therapy	Normal psychomotor development; treated as chromosomal breakage syndrome early on, although exact type of syndrome could not be distinguished promptly	–
47	NBN	homozygous 657del5	25	7	–	26.2	Severe chemosensitivity, known chromosome instability (detected at age 3) --> work up	Isolated deficiency of IgG4	n.a.	–	Chronic juvenile polyarthritis, immunosuppressive therapy since age 7. Deficiency in visual perception, determination of numbers and quantity, fine and gross motor skills as well as endurence and resilience;	B-non Hodgkin lymphoma

NBN – Nibrin

4.6 Artemis deficiency (AD)

One patient with Artemis deficiency could be included in the thesis. The patient presented with OMENN Syndrome and physical abnormalities of the urogenital tract, as well as severe combined immunodeficiency right after birth. A homozygous splice-donor mutation in c.400+1GT>TT was identified. The patient received matched-sibling-donor HSCT two months after birth. *Figure 22* shows immunoglobulin levels in the months since transplantation. The deviation of all counts towards the minimum normal range was calculated, levels above the normal range were circled in red.

The patient undergoes regular follow-ups and was alive 8.1 years after HSCT. Frequent infections as well as short stature have been described over the past years.

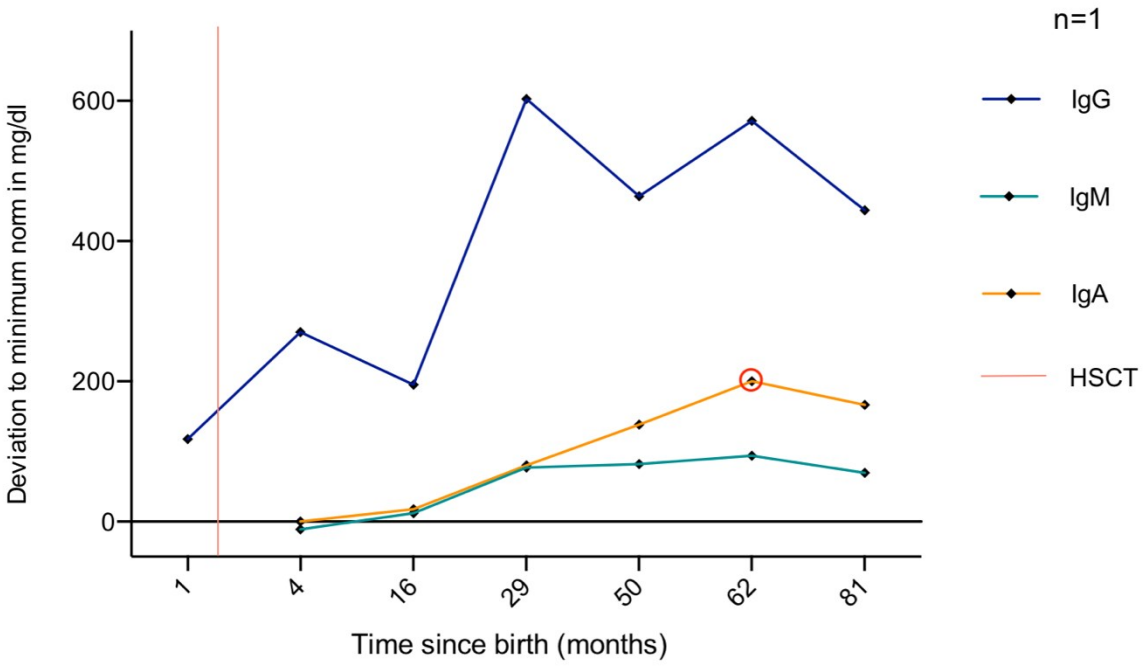


Figure 22. Artemis deficiency: Immunoglobulin levels over time. *Deviation to minimum, age appropriate norm range is shown. Pink line indicates time point of HSCT.*
HSCT – hematopoietic stem cell transplantation

5 Discussion

Studies about chromosome instability syndromes with DNA-repair defects were or are being conducted in countries like the United States, Germany, and France, among others, looking at survival, development of malignancies and therapeutic interventions. However, to date no such study has been performed in Austria. Moreover, no study looking at multiple syndromes derived from such genetic abnormalities has been done.

In this work hematologically and immunologically most relevant syndromes with DNA-repair defects in Austria were analyzed, to look at the type and frequency, the progression and treatment of these diseases, hematological and immunological manifestations, and ultimately the development and type of malignancies.

Forty-seven individuals were included with the diagnosis of either Fanconi anemia, Ataxia telangiectasia, Nijmegen breakage syndrome and Artemis deficiency. Patients with Bloom syndrome, constitutive mismatch repair deficiency, DNA ligase 4 deficiency or Cernunnos deficiency could not be identified. Most patients enrolled were treated in Vienna, which is not only explicable by the distribution of Austria's population, but also by the high density of institutions and specialists in Austria's capital city as well as referrals from neighboring countries for HSCT in FA-linked malignancies. Although no exact prevalence is known for either of the included syndromes, estimates indicate that FA is the most frequent one.[12,13,79] The distribution of patients in Austria is a reflection of these estimates and FA represented the biggest group. The male to female ratio in FA was 1:1, which is close to what was described in other studies (male vs female 1.2:1).[10] Moreover, these patients showed an earlier age at symptom onset and diagnosis when compared to other studies. It has been previously described that the onset of symptoms may be dependent on the underlying mutated complementation group. However, as a major drawback of this study, genetic results were not available for 27 of 30 FA patients.[9,33,34] Two patients for whom genetic results were available, showed mutations in FANCA in both patients, on patient had an additional mutation in FANCI. FANCA has been reported as the most frequent mutation, whereas only few cases with a mutation in FANCI are known.[13,15,145] One patient showed a mutation in FANCD1, a mutation previously described with severe phenotype and poor prognosis.[146]

Physical abnormalities were described in 92,9% of patients, a number much higher than what results from other studies report (50-70% of patients).[9,33,34] Nonetheless, short stature and abnormalities of the skin were the most common findings, just as described in a review by *Shimamura et al*.[69] Hematological abnormalities were found in all patients and two thirds received HSCT. A clear correlation between survival and transplant donor relation could be found, just as reported in literature.[144,147] The median age of patients at HSCT was 11.6 years (range, 4.2-29.2 years), which is higher than the one reported in a group of Israeli FA patients (median 9.5 years).[63] This study analyzed patients with bone marrow failure syndromes undergoing HSCT in which 66 FA patients were included.[63]

The report by *Tamary et al.* was used as a reference to compare the results of this work.[63] Transplant indication was analyzed, and AML occurred in 10% of FA patients in Austria, similar to what is described in the group of Israeli FA patients (11%). The mean age at AML onset with 12 years was slightly higher in the Austrian cohort than what the authors of the Israeli study described (onset at 10 years).[63] MDS occurred in 7 FA patients (23%) in this study. However, two of these patients were diagnosed of AML shortly after. In one patient, AML was diagnosed two days after the initial diagnosis of MDS, after a second consultation. In the other patient, findings of MDS lead to an immediate search for a transplant donor, but three months later, by the time a donor was found, MDS had progressed into AML. Thus, only five patients (16%) remained with MDS as indication for HSCT with a mean age at onset of MDS of 18.4 years and were transplanted. The same percentage of MDS as transplant indication was described in the Israeli study.[63] However, the patients in the present study had a higher age at disease onset, compared to the mean age at onset of 16 years in Israeli study.[63]

Furthermore, it must be considered that this was not a purely retrospective analysis. Most patients are alive and 8 patients remain not transplanted. The median age of this group is 7.2 years (range, 3.3-23 years), a long-term follow-up is being performed to observe the probable development of malignancies and intervene as soon as possible.

To date, head and neck squamous cell carcinoma (HNSCC) was seen in only one patient, at age 34.3 (26.6 years after HSCT). Three more patients are close to this age (29.9-31.2 years at last FU) and have been transplanted in the past. It is known that FA patients have a much higher risk of developing HNSCC than the general

population, the tumors emerging between 20 and 40 years of age. It has been shown that HNSCC may occur even 10 years earlier in transplanted patients. Thus, these three afore mentioned patients should be closely monitored to discover a possible malignancy as soon as possible.[59] One patient, the one with the severe mutation of FANCD1, died of hepatoblastoma; this patients course was previously published in a case report by *Kopic et al*.[148]

Patients with Ataxia teleangiectasia represented the second largest group. In 10 patients the male to female ratio was 2:8. This relation is higher on the side of females, than what has been described otherwise (male to female 2:3).[102] When looking at age at symptom onset and symptoms leading to diagnosis, this group of patients showed the same pattern as described in literature.[73,74,85] Also, the pattern of immunological findings was just as what has been described in previous studies, with isolated IgA deficiency, deficiency of IgG-subclasses and in the cellular compartment combined B- and T-cell deficiency as well as isolated T-cell deficiency.[87,91,149]

Micol et al. studied a cohort of 240 ATM patients, of whom 22% had malignancies, whereas 40% of ATM patients (four patients) included in this thesis developed malignancies.[150] The mean age at diagnosis of a hematological malignancy was much higher in the Austrian cohort than in the study by *Micol et al.* (19.2 *vs.* 11.4 years, respectively).[150] The rate of patients with hematological malignancies was the highest among other malignancies, in accordance with previous reports. Genetic analysis was obtained in five patients. Two related patients showed compound heterozygous mutations in c.3850delA together with base exchange mutation in c.6095G>A and one patient showed a base exchange mutation in R2443X. These abnormalities were already described in a cohort of German patients.[94] One patient showed a homozygous deletion of c.381delA, which was previously reported.[151]

Two patients showed mutations which were not previously reported in literature: a homozygous base exchange in c.4236+4A>G and hemizygous base exchange c5309C>A with heterozygous deletion of exon 34-46 (Voss and Seidel, *unpublished observation*).

The third largest group were patients with Nijmegen breakage syndrome. Five out of six patients had the most common mutation found in NBS, 657del5.[110] One

patient showed a heterozygous missense mutation in R215W, a mutation previously described in combination with 657del5 in twins affected by NBS, by *Seemanova et al.*[152] They reported a severe phenotype with psychomotor development retardation, which was not found in the Austrian patient. However, in this patient no second mutation in NBN could be identified in the entire coding sequence, yet despite a clear NBS phenotype. Furthermore, a higher risk to develop malignancy has been described in patients with the R215W mutation.[152] To this date, the patient included in this thesis with the above mentioned mutation has not developed malignancy.

Phenotype and symptom onset were found to be just like described in literature in 5 of 6 patients. Immunodeficiency regarding cellular immunity and humoral immunity was similar to what has been described in other studies.[122] HSCT was not performed in any patients, although it could be considered for patient 33, a patient undergoing immunoglobulin substitution but with severe infections nonetheless and Pseudo-hyper-IgM-syndrome. This syndrome has been described in NBS-patients to occur frequently before the development of malignancy and was suggested to be regarded as prognostic marker.[118,124] Because most neoplasms in NBS are of hematological origin, it may be speculated, that HSCT could prevent the occurrence of malignancy in the hematopoietic and lymphoreticular system. In fact, it was proposed that NBS patients with hematological malignancy should be transplanted in first remission to reduce the future risk of relapse or secondary cancer of the hematological system.[153] Studies claim that no secondary malignancies have been observed, but careful long term follow-up is needed to confirm this finding. [122,125]

Chrzanowska et al. describe the development of malignancy in 40% of patients by age 20.[22] In the present study, malignancy occurred in one of three patients aged around 20 years. This was patient 47, who developed B-non-Hodgkin lymphoma (B-NHL). As mentioned above, Hyper-IgM-syndrome has been described as a "predictor" for malignancy. For patient 47, only one serum IgM concentration could be obtained and it was substantially increased. Another patient, patient 33, age 10.8 years at last follow up, showed increased IgM levels, continuously rising over the past years. We conclude from our data and the published evidence, that this patient should be closely monitored, to detect eventual malignancy as early as possible.

Patient 47 posed a special case. This patient suffered from chronic polyarthritis and underwent immunosuppressive therapy starting at the age of 7 years. During this therapy, the patient showed no increased risk of infections. Analysis of

immunoglobulin levels showed mild IgA deficiency. In retrospective, it can only be speculated, whether this deficiency was a result of the underlying syndrome or merely a side effect of years of immunosuppressive treatment, as described in other studies.[154] Only 2 cases have been reported about a chronic-polyarthritis-like manifestation in NBS. In these reports, the underlying disease was already known at the time chronic polyarthritis was diagnosed. [155,156] Both reported patients showed signs of immunodeficiency in addition to autoimmunity.[155,156] In contrast to these reports, patient 47 showed no signs of immunodeficiency when being treated for polyarthritis, whereas the diagnosis of NBS was still unknown for many years. The diagnosis was made, when the patient developed severe chemo-sensitivity during treatment for B-NHL nearly 20 years after the primary symptoms occurred.
This case is also a good example of how often symptoms that may indicate a chromosome instability syndrome are overlooked and how definite diagnosis may take years after enough evidence is gathered, because attending physicians may be unfamiliar with these rare diseases. Patient 47 was indeed suspected to have a chromosome instability syndrome “similar to Ataxia telangiectasia” already at the age of 3 years, when cytogenetic testing showed a translocation t(7p14)(14q22) reminiscent of ATM-associated chromosomal aberrations. However, no further genetic or molecular biological tests followed, and the definite diagnosis was not made until 22 years later, when new symptoms arose.

The smallest group consisted of one patient with Artemis deficiency. It is known, that most patients with Artemis deficiency die within the first year of life. As a successful therapy for Artemis deficiency-associated severe combined immunodeficiency and Omenn syndrome as in the present patient, HSCT has been performed in several individuals so far.[157] The outcome for Artemis patients has been described as poor, with complications as high as patients undergoing transplantation with unrelated donors.[158] Other studies suggest higher rates of long term complications, such as growth retardation, endocrinological abnormalities and infections.[157,159] The patient included in this thesis developed no severe acute complications after matched SD-HSCT. Whereas hormonal status was not available, notes of delayed growth and recurring infections were found. Of note, close prophylactic monitoring for the development of future malignancies is warranted due to the DNA repair deficiency in all tissues except the transplanted hematopoietic system.

One of the limitations of this study was, that due to the retrospective nature of this multicenter analysis, and because each center collected data according to their own standards, a systematic way of collecting the relevant patient information was difficult, making data harmonization and calculation of results rather challenging. For example, genotype information for Fanconi patients was not available in 94%, either because genetic testing had not been done, or because a written report could not be found.

The small patient numbers and big heterogeneity of patients within their syndrome also posed a limitation in this study.

The inclusion criteria for this study meant that patients deceased by the year of 2000 were excluded. This was limiting when looking at the outcome of HSCT by comparing conditioning regimes. Conditioning regimes used in the early 90ies were composed of cyclophosphamide or a combination of cyclophosphamide and ATG. By the late 90ies regimes were continuously improved by adding fludarabine and combining other chemotherapeutics, after studies showing better outcome had been published.[50,160,161]

Looking at the HSCT analysis in this study, it appeared that patients in the early 90ies had better chances of survival. Only one of seven patients, who had bene transplanted in the early 90ies died (14.2%), whereas 8 of 14 patients (57.1%) transplanted after 2000 were deceased. These findings would lead to the misinterpretation, that the revised regimes of the late 90ies do not improve the outcome of HSCT only because the mortality in early 90ies was reduced by the excluded patients.

This work presents a summary of the current status of hematologically and immunologically relevant chromosome instability syndromes in Austria. On one side, it may be useful to have an overview of all these diseases to raise awareness among pediatricians. Especially because the share same features, awareness of the various clinical manifestations of such syndromes is crucial to allow earlier diagnosis. This is particularly relevant for physicians in small practices, since they usually are the first doctors confronted with such patients. On the other side, a prospective study with a systematic analysis of each disease individually, particularly of those syndromes of higher frequency, like FA, would provide an

accurate assessment of its characteristics. This is crucial not only to better understand such syndromes but to find new and better approaches to therapy.

The findings of this work are collected in a database, that serves as the basis for a future registry accessible for treating physicians within Austria. It will provide references to the course of disease and treatment and allow the long-term follow up of these patients. Furthermore, it is intended as platform for new collaborations for future studies with bigger patient numbers.

In short, the results of this work provide a reliable estimate of the prevalence and depict the clinical course and management of FA, NBS, ATM and AD in Austria. The importance of the results derives from the rarity, the heterogeneity and severity of these syndromes. Not only the early identification of affected individuals but also the appropriate, personalized treatment modifications are crucial for the survival and wellbeing of these patients. The present registry and network will enable future clinical research in Austrian patients with these orphan diseases.

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Appendix

Table 1. Fanconi anemia genes, proteins and pathologies associated with their inactivation

Gene	Synonym	Main protein functions	Gene frequency within FA patient population (%)	Symptoms	References
FANCA		Component of FA core complex; interacts with BRCA1	66	FA pathologies	162
FANCB		Component of FA core complex	2	FA pathologies	163
FANCC		Component of FA core complex	10	FA pathologies	164
FANCD1	*BRCA2*	HR repair; loads RAD51 onto DNA; interacts with FANCD2 and FANCN; stalled replication fork protection	Rare	FA pathologies; not all patients display bone marrow failure; mutation carriers have higher risk of breast and ovarian tumours and lower onset age	165–167
FANCD2		Ubiquinated after DNA damage; MCM interaction; stalled replication fork protection	2	FA pathologies	168
FANCE		Component of FA core complex; interacts with FANCD2	2	FA pathologies	169
FANCF		Component of FA core complex	2	FA pathologies	169
FANCG	*XRCC9*	Component of FA core complex	9	FA pathologies	169
FANCI		Ubiquinated after DNA damage; activates dormant origins	< 2	FA pathologies	15,145,170
FANCJ	*BACH, BRIP1*	FA repair; HR repair; 3' to 5' helicase; interacts with BRCA1; checkpoint activation	< 2	FA pathologies	171–173
FANCL		E3 ubiquitin ligase; component of FA core complex	Rare	FA pathologies, no cancers reported	174
FANCM		DNA helicase/translocase; localises the core complex to DNA; required for FANCI-FANCD2 ubiquitination; checkpoint activation	Rare	Phenotype unknown because the only patient described in the literature also has a FANCA mutation	175
FANCN	*PALB2*	HR repair, promotes BRCA2 function; interacts with BRCA1 and BRCA2	< 2	FA pathologies; mutation carriers have higher risk of breast cancer	176,177
FANCO (provisional)	*RAD51C*	HR repair; promotes RAD51 nucleoprotein filament stability; ICL repair	Rare	FA-like syndrome; patients d not thus far display bone marrow failure or cancer	178,179
FANCP	*SLX4*	Coordinates XPF-ERCC1, MUS81-EME1 and SLX1 nucleases; resolves Holliday junctions	Rare	FA pathologies	180–182
FANCQ	*ERCC4, XPF*	Endonuclease; binds to ERCC1; crosslink unhooking	Rare	FA pathologies; one patient also displayed Cockayne syndrome and xeroderma pigmentosum	183,184
FANCR	*RAD51*	HR repari; ICL repair; protection of nascent strands from DNA2- and WRN-mediated resection; stalled replication fork protection	Rare	FA-like syndrome; patients do not thus far display bone marrow failure or cancer	185,186
FANCS	*BRCA1*	HR repair; promotes Rad51 loading; ICL repair; chromatin dissociation of replicative helicase; stalled replication fork protection; interacts with FANCD2 and FANCN	Rare	FA-like syndrome; patients do not display bone marrow failure; mutation carriers have higher risk of breast cancer and ovarian tumours and lower onset age	187
FANCT	*UBE2T*	E2 ubiquitin-conjugating enzyme for FANCI-FANCD2 complex; interacts with FANCL	Rare	FA pathologies	188–191

Table 9. Physical abnormalities in Fanconi anemia n = 28*

PatID	Head	Eyes	Ears	Neck	Skin/Nails	Chest/Lung	Extremities	Sceletal	Urogenital	GIT	other
1	–	–	–	–	–	–	–	–	Pelvic kidney	–	–
2	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	Dystropic kidneys	–	–
4	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	Hyper-pigmentation	–	Foot dysplasia (clubfeet)	–	Hydronephrosis	–	Short stature
6	–	–	–	–	Café-au-lait spots	–	–	–	Hypogonadism	–	Short stature
7	–	–	Aplasia of auditory canal R; - stenosis L	–	–	–	–	–	–	–	–
8	–	–	–	–	Café-au-lait spots	–	–	–	Pelvic kidney R	–	Short stature
9	–	–	–	–	Café-au-lait spots	ASD, tricuspid regurgitation	Hypoplasia phalanx R; double thumb L	–	Retractile testis L	–	–
10	–	–	–	–	Café-au-lait spots, Vitiligo Thorax	–	Thumb anomaly	–	VUR R, intrarenal reflux; L pelvic kidney	–	Short stature
11	–	Ptosis, enge Lidspalten	–	Pterygium colli	Café-au-lait spots, Vitiligo punctata neck and trunk	–	–	Genua valga	Phimosis	–	–
12	Craniofacial dysmorphism: microcephaly, microstomy, small nose, pointy chin, misalignment of teeth	Hypotelorism, microphthalmia, hyperopia	Big, low set ears; labyrinthine deafness	–	Café-au-lait spots, hyper-pigmentation (trunk)	Vitium cordis	Thumb aplasia L + R	Elongated 1st rib L + R, 12th rib missing L;Hipdysplasia	Dystopic kidneys (pelvic), VUR L	–	Short stature
13	Microcephaly	Hypotelorisnm, epicanthus	Hypakusis	–	–	–	Thumb dysplasia R+L, hexodactyly L hand, syndactyly of 2nd & 3rd toe R+L, skew & flat feet	Skoliosis L convex	Hypoplastic kidney, horseshoe kidney	–	Short stature
14	Head dysplasia	Hypertelorism	Labyrinthine deafness L	–	Café au lait spots, hyper-pigmentation, hypertrichosis	–	–	–	Hypergonadoropic hypogonadism, ovarian insufficiency, hypertrophic clitoris	–	Short stature
15	Head dysplasia, microcephaly	Strabism	–	–	–	–	Thumb hypoplasia, phalanx aplasia L	–	–	–	Short stature
16	Microcephaly, flat nose	Epicanthic fold, ptosis, microphthalmia	Hypakusis R + L	Short neck	–	–	Thumb hypoplasia, thenar hypoplasia	–	–	Esophageal atresia	Short stature

Physical abnormalities of patients *1 -16; table continues on the next page.*

* Information could not be obtained for two patients.

ASD – atrial septal defect, L – left, R – right, VUR – vesicoureteral reflux

Table 9. Physical abnormalities in Fanconi anemia n = 28*

PatID	Head	Eyes	Ears	Neck	Skin/Nails	Chest/Lung	Extremities	Sceletal	Urogenital	GIT	other
17	Flat nose	Hypotelorism, ptosis, microphthalmia	–	–	Hypopigmentation	–	–	–	Phimosis, retractile testis L	–	–
18	–	–	–	–	–	–	Thumb aplasia, radial aplasia	–	Renal agenesis L	Duodenal atresia, esophageal atresia, esophago-tracheal fistula	VACTERL
19	–	Hyperopia	–	–	Vitiligo	–	–	–	–	–	Short stature
20	High palate	–	–	–		Dilated heart R>L	Thumb aplasia, Thumb hypoplasia	Hip dysplasia	–	Hepatomegaly	–
22	Microcephaly, distinct facies, low set ears	–	–	–	Café au lait spots, hyper-pigmentation, hypopigmentation	–	–	–	–	–	Short stature
23	Microcephaly, facial assymetry	Epicanthal folds, microphthalmia	–	Torticollis	Hyper-pigmentation (chest, back)	–	–	–	–	–	–
24	–	–	–	–	Café au lait spots	–	–	–	–	–	–
25	–	–	Hypakusis/inner ear dysplasia	–	–	–	–	–	dystopic kidney	Anal atresia	short stature
26	Microcephaly, distinct facies	Microphthalmia, Strabism	–	–	Café au lait spot, nail dystrophy	–	–	Hip dysplasia	VUR	Anal atresia	short stature
29	–	Hypotelorism, epicanthus	–	–	Hyper-pigmentation	–	Thumb hypoplasia R, clinodactyly R	–	Crossed renal ectopy, big right fusion	–	–
30	Microcephaly, low set ears	–	Atresia of acoustic meatus R + L	–	–	Hypertrophic right heart	Hexodactyly R + L	Sacral dysplasia	Renal agenesis left, crossed renal ectopy, VUR	Anal atresia	VACTERL, short stature
43	Microcephaly	–	–	–	–	–	Thumb hypoplasia L	–	–	Esophageal atresia, hernia diaphragm	–

Physical abnormalities of patients *17 – 43.*
* Information could not be obtained for two patients.
L – left, R – right, VUR – vesicoureteral reflux

Table 10. Physical abnormalities in Ataxia telangiectasia n = 7*

PatID	Head	Eyes	Ears	Neck	Skin/Nails	Chest/Lung	Extremities	Sceletal	Urogenital	GIT	other
36	–	–	–	–	Hypopigmentation	–	–	–	–	–	–
37	–	–	–	–	–	–	–	–	–	–	–
38	–	–	–	–	–	–	–	–	–	–	–
41	–	Telangiectasis	–	–	–	–	–	–	–	–	–
44	–	Telangiectasis	–	–	Café au lait spot	–	–	–	–	–	–
45	Microcephaly	Telangiectasis	–	–	Hairy pigment naevus >1cm	–	–	–	Hydronephrosis	–	Failure to thrive
46	–	Strabismus, Telangiectasis	–	–	–	–	–	–	–	–	Redness and telangiectsis back

* Information could not be obtained for three patients.

Table 11. Physical abnormalities in Nijmegen breakage syndrome n = 6

PatID	Head	Eyes	Ears	Neck	Skin/Nails	Chest/Lung	Extremities	Sceletal	Urogenital	GIT	other
21	Microcephaly	Microphthalmia	–	–	–	–	–	–	–	Rectal atresia, anal atresia	–
31	Microcephaly, high palate	–	Hypacusis R + L	–	Hypopigmentation, palmo-plantar hyperkeratosis	–	–	–	–	–	–
32	Microcephaly	–	–	–	–	–	–	–	–	–	–
33	Microcephaly, high palate, rethrognathia	Hypotelorism, mongoloid eyelids protrusio bulbi	–	–	–	–	–	–	Hydronephrosis R	–	–
34	Microcephaly	–	–	–	Café au lait spots	–	–	–	–	–	–
47	Microcephaly	–	–	–	–	–	–	–	–	–	Short stature

L – left, R – right